

Chronic Currency Crisis Continues

THE latest bout of uncertainty about the stability of the international currency system has now been temporarily resolved by yet another adjustment of parities. Next will come more concessions to the United States on the restriction of international trade and a still further complication of the rules that govern the international money market and in particular the still further elaboration of the attempt to distinguish between commercial and capital transactions of the kind now embodied in their purest form in the two-tier system operated by the French. Nobody will expect that the new arrangements will last very long. It has taken just over twelve months for the Smithsonian Agreement to collapse. But the prospects for the future are not especially bright. The restrictions on imports maintained by the Japanese government will no doubt continue, in flagrant violation of the spirit if not the letter of the General Agreement on Tariffs and Trade. The United States will no doubt be edged in the same direction by the uncertainties of the past few weeks. And there is still no sign that the European Community is in a position or even a mood to create the financial institutions without which the concept of a European Community is incomplete.

What is to be done? The first need is that there should be a concerted attack on the Japanese position. The remarkable growth of the Japanese economy in the past few years is entirely a consequence of the way in which the Japanese export trade has flourished and thus of the willingness of countries elsewhere to import Japanese goods freely. During 1972, nations such as the United States and Britain, anxious to reduce the resulting drain on their balance of payments, have attempted to persuade the Japanese government to induce Japanese exporters to adopt voluntary restrictions on their export trade, but this expedient is at once unworkable and less satisfactory than a thorough liberalization of the rules by means of which the government of Japan ensures that it remains in surplus with its trading partners. But since Japan has every interest in being in the international swim, it is surely a simple issue of international diplomacy that there should be a further and rapid liberalization of Japanese trade. Whether the result would be, as the United States would hope, an immediate rectification of the adverse balance of United States trade is however doubtful—what the Administration has to acknowledge is that the effective devaluation of the dollar in 1971 and that now in effect have been brought about by the changed balance of prosperity between the United States and the other advanced countries of the world which has become apparent in the past decade.

Where the European Community is concerned, the immediate need is to develop machinery not merely for forming a common financial policy but for broadening the base within which such a policy is worked out. At the Paris Summit meeting last year, the nine members of

the European Community paid lip service to the view that by the end of the present decade there should be a common monetary system, which implies not merely a common unit of currency but the financial institutions needed to back it up and, more important, a measure of permanent agreement on the equitable distribution of resources within the European Community. In other words, before a monetary union is possible, the member countries of the European Community will have had to agree on a common policy for the support of social services, including the arrangements for paying unemployment benefits to those who are out of work. There will have to be a common regional policy and machinery for economic planning at least in the broad sense of determining the steps which need to be taken to ensure an equitable distribution of industry and of jobs within the nine member states.

Currency crises are either calamities or opportunities according to the mood of those who must deal with them. Formidable though the obstacles to economic integration in Europe may be, one of the disappointments of the past week's negotiations is the apparent lack of an explicit recognition that the edge might be taken off the problem of currency instability by accelerating the timetable provisionally agreed in Paris for economic union. On the face of things, it would have done no harm at all if Mr Anthony Barber, the British representative at last week's Paris meeting, had advocated that the timetable for economic union should be advanced to, say, July 1974. Although there are formidable issues to be settled, what needs to be done is clear enough and the European Community has in the past made progress chiefly by setting itself apparently impossible timetables and then staying up all night to meet them. And in the present situation, anyway, the whole concept of the European Community will be incomplete until these radical issues are decided. Indeed, the arrangements which the community is trying to force on its members for the regulation of currency transactions themselves contain the seeds of further instability. Currencies are meant to move only narrowly against each other, and the British government has promised that the floating pound will eventually be fixed. But how can such a system survive the inevitable imbalances of trade among the members of the European Community if there is no mechanism for making sure that countries which run a deficit on their trading balance are somehow helped to make up the difference? It follows that the members of the European Community should hurry to settle their internal arrangements for exchanging currencies in case they find they have built into their internal arrangements the kind of instability which has now emerged internationally. It is unthinkable that the present system can survive until 1980 and the sooner European governments acknowledge that the better.



Decisions in Secret

THE Post Office believes that it has learned two lessons from its own history. The first is not to put all its eggs in one basket; the second is to disguise big decisions so that they cannot later be said to have been wrong. For the switching system for the British telephone network in the years immediately ahead, the Post Office has, after long deliberation, decided to order the controversial TXE-4 reed-relay exchange. But it will not order nearly as many as it might have done. At the same time, it will increase by tenfold its purchases of crossbar exchanges (which advanced telephone systems in other countries now rely on), so that crossbar will actually outweigh TXE-4 in the system to a considerable degree. And there will be more orders of the smaller TXE-2 exchanges which, like the TXE-4, depend on reed relays to make their electrical contacts. This mixed package will bridge the gap between the antiquated Strowger step-by-step switching on which Britain's telephones deplorably now depend and the electronic, solid-state, digital, computer-directed exchanges of the future.

This mix has been specially designed to placate the Post Office's three major suppliers, Standard Telephones and Cables, GEC, and Plessey. STC has been producing TXE-4s—16 of them under an initial contract worth £15 million. But the other two, Plessey particularly, were not keen to follow suit. They have seen the TXE-4 as a stopgap invention, eccentrically British, of little export value. The Post Office would probably have liked to nominate the TXE-4 as the standard large (over 10,000 lines) exchange for the next few years but it would have done so at the risk of offending Plessey and also of making a conspicuous major error.

But is the decision the right one for Britain? The Minister of Posts and Telecommunications, Sir John Eden, will have to give his approval before it is made final. Sir John appreciates the gravity of his action, about £1,300 million is to be spent on telephone exchanges over the next five years and the present state of the service is sad and the long waiting lists of would-be subscribers are a political embarrassment. But who will advise him—if not the Post Office and the three suppliers most concerned?

Such important choices and the debates that precede them should be made in public. The fact that the details are technical and tedious to the ordinary politician, let alone the man in the street, is irrelevant. There are a great many technically literate people around and a great number of companies (one must remember that they need not all be British) which might have technical and commercial solutions to offer on Britain's telephone dilemma. Their views should be heard. Sir John Clark, chairman of Plessey, worked hard to dissuade the Post Office from too heavy a reliance on the TXE-4 and apparently he succeeded. But should not such experts from vested interests give their advice to the Post Office in the knowledge that it will be open to criticism from those outside the Post Office?

The uncertainties over the TXE-4 are not hard to resolve. People at STC are confident that it has export potential, that it is the best possible bridge between Strowger and an all-electronic system and that it can be modified, as the telephone network is modernized, to in-

clude solid state technology. But had the deliberations been open, people might have been reminded, as they certainly have not, that the TXE-4 will not give telephone users any of the new services like abbreviated dialling, calls recorded at the exchange if the subscriber is out, and automatic transfer to calls to another number that are becoming available in Canada and the United States.

The TXE-4-crossbar compromise has been secretly arrived at and it has done little to create the needed new relationship between the Post Office and industry on research and development. Moreover, it seems to have been conducted with hardly a mention of the word Europe, even though the European Economic Community intends that there should be an all-European integrated telephone network by the 1990s, with a true common market in supplies of communications equipment.

Euratom Soldiers On

THE best thing to say about the agreement between the European governments reached in Brussels on February 6 about the future of Euratom is that it provides a tangible programme for the next three years. Uncertainty is good for nobody and the uncertainty which has plagued the four joint research laboratories for almost five years has been especially damaging to the staff. So far, so good. But it remains something of a mystery to know whether the compromise at Brussels will do much more than postpone the question of what should be done about the four laboratories which are in practice the only tangible legacy of the ambitious plans for a coordinated European research programme in nuclear energy mapped out by Euratom in the 1950s. Where nuclear fission is concerned, the joint research laboratories have only an insubstantial contribution to make to the development of practical power stations—their experimental and test reactors, for example, contribute nothing of substance to the devices which are available elsewhere, while there is no prospect that the joint research laboratories will be able to recapture the opportunity for coordinating development on schemes such as those for fast reactors now being pursued independently, and with very little collaboration, in Britain, France, Germany and Italy. On fusion, the laboratories have a potentially more valuable part to play, but even here the sceptics can fairly say that even this opportunity will be lost before another three years have passed.

What might be done? A coordinated policy on fusion research is necessary and it would be in everybody's interest if the several governments with a finger in the pie would at least explore the possibility that Euratom might become a coordinating authority. At the same time, however, it might be recognized that Euratom has very little more to say or do about fission reactors, but that there is a need—an urgent need—for a better articulation of national research programmes on fast reactors and that Euratom might act as a kind of secretariat for that process. For the rest, it should be acknowledged that the best use of the staff that Euratom has accumulated would be to serve as extra manpower for the national laboratories already doing useful work in fields where European integration is inevitable—in standards and metrology, for example. The fact that the new budget

for Euratom does provide for the transfer of Euratom staff to national laboratories is in this sense an advantage. In the period between now and April, when the European Commission is required further to define the research programme, and in the two years between now and the next review of the programme, urgent steps should be taken to see that schemes like this are thoroughly explored.

Pipeline in Trouble

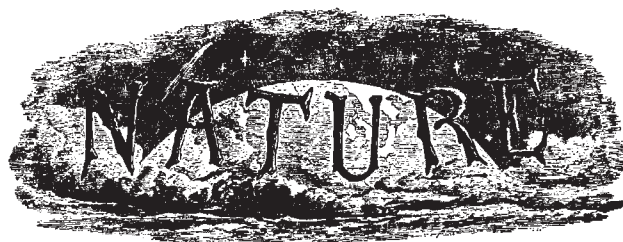
THE chance that the United States will be able to reap the benefits of the vast oilfields on the North Slope of Alaska continues to recede. A week ago, the United States Appeals Court astonished all parties to the project to build a pipeline from the North Slope to Prudhoe Bay—the consortium of petroleum companies, the disputant environmental groups and no doubt the US Department of the Interior as well—by ruling that the department has no authority to grant a permanent right of way across federal lands to those who would have to service any pipeline that might be built. It is, of course, astounding that the Department of the Interior should have been caught out on a technical legality like this. The result will be that if the department can summon up the nerve, Congress will have to be invited to change the law, in circumstances in which it will be impossible to separate the strictly legal argument from the wider environmental arguments which have bedevilled the Alaskan pipeline almost from the start. Although it is quite fair that complainants should have protested, at the beginning, that the petroleum companies were willing to push ahead with the pipeline project with only scant concern for environmental considerations, more recently it would have been more proper to complain that the Department of the Interior has been a pusillanimous arbitrator in the dispute, and reprehensibly slow to give a lead.

What should it now be urging? The legal argument quite apart, the urgent need is for the department to decide just what contribution the North Slope can be expected to make to the supply of energy in the United States. And if, three years ago, the department's opinion on the virtues of the pipeline project was evenly balanced—the more charitable interpretation of its equivocation—the recognition of the petroleum shortage in the past six months must surely have tipped the balance in favour of exploitation. Surely it will be a strange situation if the United States now shoulders the burden of paying up to \$50,000 million a year for imports of oil from the Middle East and at the same time decides to leave the petroleum beneath the North Slope where it is. It will be stranger still if the United States permits an increase in the price of petroleum, which could easily double in the next few years, while imposing on itself a self-denying ordinance where Alaskan oil is concerned. Nobody will pretend that the environmental arguments against the Alaskan pipeline should be brushed aside, but at some point in the next few months the United States will have to recognize that some at least of what it now calls the energy crisis is the price which it has chosen to pay for leaving Alaskan oil alone.

At the same time, the United States must somehow come to grips with the problem of putting a value on the

environmental qualities which it has come to revere. A part of the argument against the pipeline is that it will permanently disturb a unique ecological system, and that is a point that does require further investigation and consideration. At the same time, a part of the United States environment to which many American voters are attached is that part of the social fabric which provides them with warmth in the winter, air-conditioning in the summer and the power needed to keep American industry in being. An accurate decision about the Alaskan pipeline requires not merely that there should be an investigation of what the environmental damage will be, but an open public discussion about the damage should be weighed against the cost either of importing fuel from the Middle East or of doing without it. Unhappily, there is no real assurance that Congress will be the right forum for making this choice.

100 Years Ago



Inherited Feeling

THE remarkable case of an inherited feeling of dislike for a special class of persons, communicated by Mr. Darwin, appears to me to support a view I have long held (but not yet published) as to the explanation of another class of so-called instincts. The three separate instances given in which the dogs showed a violent antipathy to butchers, either without seeing them or when they were dressed as gentlemen, clearly indicates that it was through the sense of smell that the painful sensation was experienced; and this is quite in accordance with the wonderful delicacy and importance of this sense in most animals, and especially in dogs. It is natural to suppose that some ancestor of these dogs was systematically and cruelly ill-treated by several butchers, perhaps from some thievish propensity or other bad habit which required frequent punishment, so that the smell of a butcher came to be invariably associated with pain and a desire for revenge. But the most important fact to observe is, that there must be some peculiar odour developed in human beings by constant contact with flesh, which a dog can recognise apart from individual peculiarities and in spite of perfect disguise. Now the power many animals possess to find their way back over a road they have travelled blindfolded (shut up in a basket inside a coach for example) has generally been considered to be an undoubted case of true instinct. But it seems to me that an animal so circumstanced will have its attention necessarily active, owing to its desire to get out of its confinement, and that by means of its most acute and only available sense it will take note of the successive odours of the way, which will leave on its mind a series of images as distinct and prominent as those we should receive by the sense of sight. The recurrence of these odours in their proper inverse order—every house, ditch, field, and village having its own well-marked individuality—would make it an easy matter for the animal in question to follow the identical route back, however many turnings and cross-roads it may have followed. This explanation appears to me to cover almost all the well-authenticated cases of this kind.

ALFRED R. WALLACE

I HAVE a cat, of a long-haired breed, whose aversion to dogs is unusually strong. Last autumn, six kittens of hers, under two days old, were in a corner of the kitchen where they had had no opportunity of making acquaintance with any dog; yet, on being stroked (in their mother's absence) by a hand which a dog had just licked, more than one of them "swore" violently. This was repeated several times, but the little creatures showed no dislike to being touched with a clean hand.

A LOVER OF ANIMALS

From *Nature*, 7, 303, February 20, 1873.

OLD WORLD

Further Support for Post-Apollo Project

BRITAIN and the Netherlands have joined the post-Apollo programme. In the past week both countries have agreed to join the European Space Research Organization's studies of the post-Apollo sortie lab and ESRO has awarded the final study contracts.

Announcing Britain's belated entry to the fold, Mr Michael Heseltine, Minister for Aerospace and Shipping, said that he had offered ESRO a contribution of £300,000 to the phase B 2 studies. Work on this, the final stage of ESRO's three part study of the technical and financial requirements of the sortie lab, start this month and will run until August. The United States has set mid-August as the deadline for ESRO to reach a decision on whether it is actually going to build the lab for NASA.

But Mr Heseltine made it clear that Britain's commitment is limited. "The UK is in no way committed to participate in further stages of the studies or in subsequent development and construction stages," he said in parliament last week.

In fact Britain has offered ESRO ten per cent of the phase B study costs, or £300,000, whichever is the smaller. The contribution is effectively backdated as it covers the phase B 1 studies which began in November and ended last month. The total cost of phase B is put at 7.5 million units of account (about £3.1 million).

Meanwhile ESRO has announced that Erno VFM-Fokker and MBB, two West German companies, have been awarded contracts worth 1.4 million units of account each, to handle the phase B 2 work.

It is a condition of the contracts that the companies sub-contract so that each participating states receives a proportion of the work related to its contribution to the programme.

Six of ESRO's nine member countries have now agreed to participate in the studies. The original four, Italy, Belgium, Spain and West Germany, agreed to foot 20 per cent, 4 per cent, 3 per cent and the remainder of the bill respectively. The addition of Britain and the Netherlands will reduce West Germany's share of the cost from 73 per cent to about 60 per cent.

ESRO, now that its "special project" to develop the sortie laboratory is established (see *Nature*, 241, 302; 1973), is committed to developing it, although the individual countries (witness Mr Heseltine's statement) are not. There is, however, an escape clause which states that the organization can withdraw if by mid-August it finds that the cost of the project will "unacceptably exceed" the original rough estimate of \$250 to \$300 million. Care has been taken not to define what "unacceptably exceed" means.

The object of the final studies is, as far as possible, to fix the costs and to "establish the configuration (of the unit) in the light of user requirements". The laboratory is expected to consist of two parts. A pressurized manned laboratory and an external unpressurized instrument platform, known as the pallet, intended for research activities on shuttle missions lasting seven to thirty days.

Now that the dust has settled on the great debate on reorganization of government research and development, Sir Alan Cottrell, who was at the heart of the debate, attempted last week to put it in its proper perspective.

Research and development, said Sir Alan at the annual dinner of the Parliamentary and Scientific Committee, is "only a small part of the total scientific and technical activity of the country". Sir Alan cited the scientists who are in management and sales, the weather forecasters, operational analysts and many others who provide scientific services to the government, as being part of the government science machine, but who were not directly affected by the green and white papers.

Britain has had many scientific and technical successes recently, said Sir Alan, the advanced passenger train, new techniques of weather forecasting and the development of equipment for the dispersal of oil at sea being just a few examples.

But now is not the time for Britain to rest on its laurels and closer understanding is needed between the researcher and the user, which, said Sir Alan, "is one of the reasons for our reorganization".

But Britain's efforts must not be spread too thinly—the requirements boards, for one thing, will ensure that this does not happen—and "we must . . . not embark on glamorous and costly developments simply because

they are fashionable". The answer in certain instances will lie in collaboration with our partners in the European Economic Community.

One of the basic problems that Britain faces is that of generating sufficient investment capital—a problem which is likely to become more severe in the coming years. But massive investment brings other difficulties, said Sir Alan, the major ones being possible inflation and balance of payments problems.

There is no worry, in the scientific sense, that material resources will be exhausted, but the price will increase, says Sir Alan. There will, therefore, be a need for improved techniques of mining and scrap recovery, and recycling will have to be intensified, all of which will need considerable scientific and technical effort. The critical factor however, according to Sir Alan, will be the availability of energy to carry out the work.

Britain is well blessed at present with adequate coal reserves, North Sea gas and oil, and nuclear developments. But Sir Alan feels that this is no time to feel complacent, and both the coal and nuclear industries must plan vigorously for the future. Parallel efforts must also be made to use energy resources more efficiently and economically.

The full text of Sir Alan Cottrell's speech will be published in *Nature* soon.

SCIENCE POLICY

Research after Rothschild

CLUB OF ROME

Onwards Undaunted*from La Recherche*

IN spite of severe criticisms since publications of *The Limits to Growth* last year, the Club of Rome, which commissioned the report from Professors Jay Forrester and Dennis Meadows at MIT, is undaunted and embarking on several more projects designed to study other related problems, particularly those of population, natural resources, and economic growth.

At a meeting held near Paris recently, the Club unfolded its seven point current research plan. The club, true to form, is, with the support of the Volkswagen Foundation, studying the effects of doubling the world population. A second group of projects surveys the reallocation of the world's resources (with emphasis on energy, food and raw materials), and also looks at the position of Japan in world affairs. A third measures the limits to German growth, the nature of change in technical innovation and the patterns of economic development in West Africa, India and the Republic of Korea.

The rest of the study areas concern national population strategies, aspects of worldwide technological forecasting and the goals and constraints which could affect future science and technology.

A group of researchers headed by Mihajlo Mesarovic (Case Western Reserve University) and Eduard Pestel (Technische Universität, Hanover), is elaborating a computer-aided "strategy for survival", a long term multi-level world model project. The group has recently published *An Interactive Decision Stratum for the Multilevel World Model*, which is largely an econometric exercise in forecasting allowable levels of consuming raw materials and other resources. To answer criticisms made of such forecasts—that variables, representing political, social or psychological decisions and events are missing — Pestel and his team will next attempt to quantify such factors for application to future models. This summer they hope to provide an analysis of future options in using the world's energy resources, and in a year's time they hope to have a model able to absorb the parameters of monetary erosion as it affects capital investment in the energy field. Pestel claims that this will lead to "new sets of decisions possible in the attenuation of the energy crisis and the polluting character of some present energy sources, as well as economic measures to be implemented".

Peccei, faithfully defending the club's philosophy of forecasting based on

computer models, said at the meeting that "Our efforts are still too modest at the Club of Rome. The first research project we did cost exactly what all the world's military forces were spending at that time in the incredibly short span of 30 seconds!"

ATOMIC ENERGY

Krypton Needs Limiting*from a Correspondent*

MEANS will have to be found to control the discharge of gaseous krypton-85 from nuclear power stations before the end of the century. Tritium release will also have to be controlled, particularly where local conditions are adverse, but otherwise the problem of an increase in background radiation from the use of nuclear power seems to be well under control. This was one of the chief conclusions of a conference on nuclear energy and the environment held in Liège recently under the auspices of the Association des Ingenieurs Electriciens. The meeting agreed with other conferences' findings that by the year 2000 the addition to the background radiation will be less than one per cent of present levels given the current projections of the increase in nuclear power plant.

The conference also discussed the problems of heat dispersal from power stations and came to the conclusion that by the end of the century Europe's rivers and lakes may not be able to absorb all the waste heat generated. The problem, it was felt, is acute, and must be tackled not only by the designers of power stations, but also by the politicians who plan energy programmes.

The International Commission on Radiological Protection (ICRP) was praised for its work in setting permitted levels of radiation early in the development of nuclear power.

Professor Z. M. Bacq, a biologist from the University of Liège, discussed the evidence that certain cells appear to have repair mechanisms that are effective in the face of low doses of radiation. This implies that there may be a threshold value below which radiation causes no cell damage. Dr F. D. Sowby, scientific secretary of ICRP, said that he was aware of this evidence that decreased doses of radiation do not produce a linear decrease in radiation damage, but he emphasized that until it is more clearly shown that the non-linear effect applies to man (to date it can only be uncertainly demonstrated in mice), the commission will continue to base its permitted exposure levels on the more pessimistic assumption that cell damage does decrease linearly with decreased radiation.

COMPUTERS

Eyes East

IN spite of government enthusiasm for a European computer company, ICL appears to have its sights set on a Japanese rather than a European link.

International Computers Ltd, Britain's largest manufacturer, told the Select Committee on Science and Technology last week that the "ever growing strength" of the Japanese industry worried it. Cooperation with Japan might be possible, however, through Japan-ICL, a subsidiary of the British company, which at present sells non-computer equipment such as punch card sorters and output punchers in Japan.

On cooperation in Europe, the company said that "in view of ICL's evident technical strength and market share relative to the other European computer firms, any unification of the industry could most effectively be centred around ICL".

The company emphasized that any benefits that are derived from harmonization of the European computer industry must be commercial in nature, and not political. But the company was not enthusiastic about the possibilities of successful links being formed. It went on to say that "it is very doubtful in ICL's view if the benefits (resulting from recent agreements on cooperation between Siemens, CII and Philips) will be sufficiently timely or substantial to have any major impact on the relative position of the European computer industry vis-à-vis United States manufacturers".

Talks between ICL and Nixdorf in West Germany and CII in France have failed to produce agreements on joint development work because of "various technical and commercial problems". Exploratory talks with United States manufacturers had seemed, ICL said, to have offered "at this point of time no worthwhile commercial advantage".

ICL reaffirmed its belief that by 1976-77 it should "have reached a level of size and profitability adequate to make further research and development support unnecessary". (The government made ICL a £14 million loan last August to help develop the company's new product line.) But the company emphasized that when this support ends the government should continue to provide development contracts for specific projects as well as orders for ICL equipment.

The company's situation has not been helped by a sharp cut in computer orders from the government. In 1971 the government announced that it expected to place orders worth £20 million with ICL that year, but up to September 1972 the company had only received orders worth £18.5 million.

COMPUTER DESIGN

Closer Collaboration

YET another call for university research workers and industrial scientists to exchange views and ideas is made in the Science Research Council's Engineering Board's report on *Research Support for Computer Aided Design* published recently.

Industry spends more than £1,000 million on design every year and the Department of Trade and Industry's computer aided design centre has estimated that a twenty per cent saving can be made in a wide range of engineering applications if the engineering industry turns fully to computer aided systems. The actual annual saving would be higher than the calculated £200 million because a switch to computer design would lead to new concepts and improved manufacturing procedures.

The report pinpoints several areas where industry would benefit from greater use of computer aided design. Traditional skills are dying in the die mould and pattern making industries and several companies are in need of new design and manufacturing techniques. The ship design industry is also a prime contender for the application of computer aided design research and the machining systems industry, according to the report, needs coordination of machine tool design, interactive aids and numerically controlled machine tools so that the industry can move more quickly into manufacturing.

The civil engineering industry is well advanced in the techniques of using computers for designing engineering systems and in integrating design products but the SRC feels that it would be prudent for the industry to now consider integrating design and production, in particular, the report says, so that selection of components, ordering of

materials and the organization of work on site should be more closely integrated.

But the introduction of these advanced computer ideas into industry needs a flow of information from universities where the basic work is being done. University workers, says the report, "should be made aware that successful contact with industry requires a considerable degree of the researcher's personal time" and it goes on to say that the best means of exchanging ideas is by personal contact, provided it is supported by "adequate and appropriate documentation". Engineers from industry should be encouraged to join university research groups for periods ranging from a week up to a year.

Research in computer aided design would also benefit from a closer knitting of existing groups, especially as the subject calls out for more interdisciplinary cooperation. The research groups should, naturally, have active support from computing science departments and should be active in running post experience courses. But the report does not favour these groups having their own large computers and they should, if the need arises, install terminals connected to a central facility.

The SRC is prepared to support groups setting out to do research in computer-aided design in engineering but the report points out that SRC grants are primarily intended to supplement existing research projects. After a suitable interval the university, or institution, is expected to assume full, or partial, responsibility for the group.

WEATHER RESEARCH

US/USSR Cooperation

from our Soviet Correspondent

A JOINT Soviet-United States research expedition to the Bering Sea was inaugurated on January 31, 1973, with the sailing of the Soviet weather ship *Priboi* from Zolotoi Rog bay, near Vladivostok. In addition to the *Priboi*, the Soviet team is supplying an Il-18 aircraft equipped as a flying laboratory, while from the American side NASA is providing an icebreaker and an aircraft.

A two-month research programme is planned, which will study the surface of the sea in various temperature conditions, the physical and chemical parameters of sea water, the density and growth of ice and the content of water vapour in the atmosphere. Measurements made from the ships will be compared with data on microwave reflexion from the sea and ice surfaces, recorded by the aircraft.

The programme has been designed under the terms of the 1972 agreement between the Soviet Union and the United States on cooperation in the

exploration and use of outer space for peaceful purposes; although no specific mention has been made of satellite data, it would seem that the readings obtained by the Bering expedition will also be compared with those of weather satellites. The material gathered by the expedition is intended for use in the development of weather forecasting methods for Arctic and sub-Arctic waters.

CRIMOND TERMINAL

Surveys Continue

TOTAL OIL MARINE is undertaking further surveys of the east coast of Scotland to examine alternatives to the Crimond site for the gas processing plant needed to handle the output of the Frigg gas field.

The disused airfield at Crimond on which Total and the British Gas Corporation want to build the processing plant lies alongside Loch Strathbeg, which is classified as of grade one scientific importance by the Nature Conservancy (see *Nature*, **240**, 435; 1973).

At a meeting in the Buchan village of Crimond last week, Mr David Wood of Total revealed that the coast had only been examined in detail from Fraserburgh to Rattray Head—a distance of a little over eight miles—but added that further surveys are in progress. This week Mr Wood said that "we are taking a complete look at the whole thing, both offshore and elsewhere". Results, he said, "are expected in weeks rather than months".

The reason for continued surveys, Mr Wood says, is that the Ministry of Defence already has planning permission to use the land for a radio station. If and when the decision on who is to have the land comes to a head "there is no guarantee that energy will get it over defence".

Mr Wood also concedes that it is likely that only four pipelines could be brought ashore at Crimond. Environmentalists fear that, in spite of the Gas Corporation's policy of concentrating processing plants on as few sites as possible, this might result in a second terminal being built elsewhere if a number of large gas fields are found in the North Sea.

Mr Wood says that he is not surprised by the reaction to the proposal, but he is surprised "that so many people think that they should be consulted first" and that people are "afraid that the local planning authority is likely to produce a *fait accompli*".

Mr Wood remains confident, however, that the construction of the terminal "will have no effect upon Loch Strathbeg whatsoever". "But getting the pipes to the airfield", he added, "that may be another matter".

Nuffield Trustees

THE Nuffield Foundation, which last year awarded more than £1 million in grants, will have a new chairman managing of trustees in April. Sir Geoffrey Gibbs, who has been at trustee since the foundation was set up in 1943 and chairman since 1951, is retiring and will be replaced by Lord Todd, who has been a managing trustee since 1950. Professor Hans Kornberg, who holds the chair of biochemistry at the University of Leicester, was made a trustee of the foundation on January 1 and will fill the breach caused by Sir Geoffrey's retirement.

NEW WORLD

More Ammunition for SST Foes

by our Washington Correspondent

WHILE Concorde was still reeling from the double blows dealt by PanAm and TWA, which decided last week not to take up their options, a committee of the National Academy of Sciences has given more ammunition to the anti-SST forces. Charged with the task of examining the biological consequences of increased ultraviolet radiation reaching the Earth's surface, the committee has concluded* that "sufficient knowledge is at hand to warrant utmost concern over the possible detrimental effects on our environment of the operation of large numbers of supersonic aircraft".

Ultraviolet radiation first burst into the SST debate in 1971, just before Congress voted to block development of two prototype American SSTs. It was then suggested, chiefly by Professor Harold Johnston of the University of California at Berkeley, that oxides of nitrogen emitted into the stratosphere by SST exhausts would partially destroy the layer of ozone in the upper atmosphere. Since the ozone layer filters out some of the ultraviolet radiation coming from the sun—particularly short wavelength ultraviolet radiation—the result would be increased ultraviolet intensity at the Earth's surface which in turn would lead to increased incidence of skin cancer and other biological damage.

Dr Johnston's calculations (*Science*, 173, 517; 1971) sparked off a scientific debate which is still essentially unresolved—in spite of a suggestion put forward in a letter to *The Times* by Sir Peter Masefield, Chairman of the British Airports Authority, which was published on January 27. Masefield stated that Johnston has admitted making a mistake in his original calculations which essentially invalidates his conclusions. Johnston said last week that such a suggestion is "quite incorrect" and that far from recanting, he has further refined his calculations and still considers his original publication a good first-order approximation.

The NAS committee, which met under the chairmanship of Dr Kendrick C. Smith, of Stanford Medical School, in spite of the general conclusion quoted above, tries to steer clear of commenting on the validity or other-

wise of Dr Johnston's calculations. It simply explores their biological implications, and in so doing, brings out several points which have previously been given scant attention.

The committee starts from the premise that a small decrease of ozone concentration in the stratosphere would produce a disproportionately large increase in ultraviolet radiation reaching the Earth's surface. Particularly affected would be the amount of radiation penetrating the ozone layer with wavelengths between 286 and 320 nm, which the committee points out are "the most biologically damaging wavelengths of sunlight". A 5 per cent reduction in stratospheric ozone at 40°N—the mid-point of the United States—would increase the intensity of ultraviolet radiation at 297.5 nm by 26 per cent, while a 50 per cent decrease in the ozone layer would cause radiation at this wavelength to increase by a factor of ten. "This point", the committee suggests, "is of the utmost importance in considering the likely biological effects that might result from man-made changes in atmospheric ozone concentrations".

But since the intensity of ultraviolet radiation is reckoned to be about 50 times greater at the Equator than it is

inside the Arctic circle—the difference is caused by a thinning of the ozone layer and increasing solar altitude towards the Equator—would a small intensity increase of about 25 per cent really be significant? The committee believes that it might, because not only is an average increase in exposure likely directly to affect man, but it may also be detrimental to plant life.

So far, the debate about SSTs and the environment has concentrated chiefly on the politically sensitive cancer aspect, but the NAS committee raises a number of much broader questions. It points out, for example, that ultraviolet radiation damages the DNA in all living things, but the damage is repaired by a variety of biochemical processes. The damage and repair are in delicate balance, which could be upset by an increase in average exposure to ultraviolet radiation—"if the amount of ultraviolet radiation damage exceeds the cell's capacity to repair this damage . . . then the cell will die". Moreover, since many plants and animals reduce their exposure to sunlight by behaviour responses triggered by heat or light, they may not be responsive simply to an increase in the ultraviolet component of sunlight.

Because ultraviolet radiation can

AEC

Woman in the Chair

by our Washington Correspondent

PRESIDENT NIXON has finally filled one of the many top-level science positions in Washington that have been vacant as a result of reshuffling, firings and resignations that followed his re-election. As expected, he has appointed Dr Dixie Lee Ray to be chairman of the Atomic Energy Commission in place of Dr James Schlesinger, who has been made director of the CIA. A marine biologist who was appointed as an AEC Commissioner last year, Dr Ray has greeted her appointment by thanking Nixon for the honour and vowing to carry on the "forward progress" which Schlesinger fostered at the agency. Otherwise, she has kept a low profile during her six months as commissioner and has thus given few clues to her thinking on nuclear matters.

Before becoming an AEC Commissioner, Dr Ray was director of the Pacific Science Center in Seattle and associate professor of zoology at the

University of Washington; her specialty was marine vertebrates. This background was expected to make her particularly receptive to environmental concerns in the commission, but so far she has made no public statements on environmental matters.

Described by her colleagues as a strong and even forceful personality, Dr Ray faces a challenging period as chairman of the commission. Not only will she have politically sensitive problems of nuclear safety and the environment to deal with, but it is likely that she may preside over the dismantling of the agency itself. The plans for reorganizing the federal bureaucracy, which President Nixon submitted to Congress two years ago, and which he has promised to resubmit this year, would have transferred the AEC's civil nuclear power functions to the proposed Department of Natural Resources. Such a move would make the commission little more than a weapons supply agency for the Department of Defense; what it would mean for the high energy physics laboratories has never been made clear.

**Biological Impacts of Increased Intensities of Solar Ultraviolet Radiation*, available from the National Academy of Sciences, 2101 Constitution Avenue, Washington DC 20418.

penetrate water to depths of a metre or more, the committee suggests that the effect of increased ultraviolet intensities on aquatic plant and animal life should be carefully studied, especially in view of the importance of plankton in the food chain.

As for skin cancers, the committee points out that the three most common malignant skin tumours—squamous cell and basal cell carcinomas and malignant melanomas—are all correlated with exposure to sunlight, and quotes a calculation that “a 5 per cent decline in ozone would produce at least 8,000 extra cases of skin carcinomas and melanomas per year in a population the size of the white population of the United States”.

One conclusion stands out clearly from the report, however—that the experimental basis for the committee's concerns is scanty, to say the best. The committee has therefore recommended, first, that a network of ground level stations should be established at various latitudes to monitor the intensity and wavelength distribution of solar ultraviolet radiation, to monitor possible environmental changes but also to enable proper evaluation of data on latitudinal variations in the incidence of skin cancer in man. Second, laboratory and field studies of the effects of increased ultraviolet radiation on the growth and yield of agricultural plants and on the sensitivity of plankton are urgently required. Finally, it is implicit in the report that these studies should be carried out well before a large SST fleet ploughs through the stratosphere.

ENERGY

Congress Committee

by our Washington Correspondent

THE House Committee on Science and Astronautics has decided to establish a new subcommittee on energy. The chairman will be Representative Mike McCormack, a second-term Democrat from Washington State, who by-passes several more senior committee members to take charge of the committee. McCormack, who until this year was the only working scientist to sit in Congress, was chairman in the last session of a task force on energy of the Science and Astronautics Committee, the final report of which is due to be published in the next few weeks. The new subcommittee is essentially an upgrading of the task force.

Although plans for the subcommittee have not yet been fully worked out, it will probably concentrate on the research and development aspects of energy production, and it will thus take a longer term view of energy supply. Some fourteen Congressional committees now have an interest in energy.

BUDGETS

The Battle Continues

by our Washington Correspondent

THE battle between President Nixon and Congress over which branch of the government should control the federal pursestrings reached boiling point last week. The focus of the disagreement is the fact that President Nixon has slashed federal spending this year by refusing to allocate some money appropriated by Congress, an action which has been regarded on Capitol Hill as usurping Congress's constitutional right to control federal spending. The White House, on the other hand, has stoically maintained that it has the authority, under a variety of acts of Congress, to apportion all or part of appropriated funds as it sees fit. The interest of the scientific community in the affair is that about \$600 million of the impounded funds were earmarked for scientific projects.

Representative John Davis, the chairman of the House Science, Research and Development Subcommittee, last week stepped into the fray by introducing an authorization bill for the National Science Foundation which is designed both to increase the NSF's budget for 1974 and to give Congress more control over the way in which it is spent. Specifically, Davis's bill breaks the foundation's budget down into a number of items and stipulates that if any money is withheld from the total budget for the foundation, it must be taken from each item on a *pro rata* basis. Such a requirement would prevent a repetition this year of the familiar pattern, in which Davis's committee votes to increase the NSF's budget for education and graduate student support, only to have the Office of Management and Budget withhold that specific increase and effectively wipe out Congress's attempt to stamp its own priorities on the budget. Davis's proposed formula would, of course, leave congressionally determined priorities intact, even though funding for each item might be reduced.

In other developments related to the power of the purse, a special subcommittee of the Senate on the Separation of Powers provided a forum for several shouting matches between senators and members of the Administration, but produced little of substance. Congress also decided to opt for a head-on clash with President Nixon over his impoundments by directing him to spend all of the \$225 million he has withheld this year from the Rural Environmental Assistant Program. All indications are that Nixon will veto the bill, and since it was passed in the House of Representatives by 251 votes to 142, it will need to pick up more support to get the

two-thirds majority needed to override the veto.

A more promising development was the publication of an interim report by a special joint committee set up last year to devise a plan for Congress to set its own budget ceiling. The committee has suggested that bipartisan units should be established in both houses of Congress, with representation from appropriations and taxation committees. The idea would be for the committees to draw up spending ceilings and revenue raising targets early in the sessions, and that the appropriations committees would work to them. The machinery for implementing such a proposal will take several weeks to work out, however, so next year's budget will have to be set in the usual manner.

ENERGY POLICY

AEC to the Fore

by our Washington Correspondent

THE Federation of American Scientists has suggested that the Atomic Energy Commission should broaden the base of its activities and become the chief agency in the federal government concerned with energy policies. The commission already has the authority for certain non-nuclear operations, the federation says, and “it is self-evidently the strongest of the government agencies for this purpose”. The idea would be to transfer to the AEC energy-related programmes of other federal agencies, to turn the Atomic Energy Commission into an Energy Agency. The FAS has felt moved to recommend such a reorganization because the so-called energy crisis is so acute that it cannot wait for longer-term bureaucratic solutions.

President Nixon's reorganization plans call for a Department of Natural Resources to be established by amalgamating several existing agencies, including those having responsibility for energy production, but the FAS points out that the plan has been languishing in the committee rooms of Capitol Hill for two years, and stands little chance of being speedily accepted this year. Broadening the activities of the AEC would, however, not require massive government reorganization.

Part of the plan would also be to divorce the AEC's regulatory responsibilities from its promotional responsibilities by setting up a single energy regulatory agency with responsibility for controlling nuclear energy, gas, oil and coal.

The plan is, however, unlikely to find friends in the White House, because President Nixon is expected soon to propose again the creation of a Department of Natural Resources.

NEWS AND VIEWS

Continuing Classification of Research

THE article on page 449 by Dr F. Winterberg is interesting in itself but it is also the tip of a large, awesome and unnecessary iceberg. What Dr Winterberg does is to calculate the possible usefulness of small fission explosions, induced with the help of a powerful beam of laser light, as a means of triggering off thermonuclear explosions in a surrounding envelope consisting of a mixture of deuterium and tritium. His article includes, in passing, an expression for the critical mass of a quantity of fissionable material—an unusual occurrence in the published literature but an expression which is now thoroughly familiar among physicists in ordinary conversation. The object of the exercise is to demonstrate that it may be more efficient to bring about small releases of thermonuclear energy by using a microfission explosion as an intermediary than to attempt to use a laser pulse to compress a thermonuclear mixture directly with the help of a laser beam. In a sense, the fissile material, because of its high density, can act as an amplifier of the laser pulse, releasing a pulse of neutrons which can directly play a part in the thermonuclear process. Nobody can tell, of course, from Dr Winterberg's account, whether his calculations would be borne out in practice. Engineers are quite properly fond of pointing out that a simple calculation like this is bound to be hypothetical in some degree. But everybody will agree that calculations like these are at once interesting and stimulating and there is much to be said for making sure that they are published.

This is why it is disturbing that in the past few months, there has grown up a cloud of officially inspired reticence about publication in the field in which Dr Winterberg works. It is widely known that the use of lasers as a means of stimulating nuclear explosions, fission or fusion, has in the past few years been considered seriously by the United States Atomic Energy Commission and similar organizations elsewhere. That is as it should be. But the US Atomic Energy Commission is also exceedingly sensitive about the publication of results in this intriguing field and, to the extent that practical applications may be concerned, that too is proper. The trouble is that the passion for secrecy which is properly applicable to the potentially practical applications of these new technologies is, of course, entirely inappropriate where theoretical considerations are concerned, especially when their authors have no official connexion with the Atomic Energy Commission. This is why it is a considerable scandal that there have recently been difficulties in refereeing articles submitted in good faith to journals, especially where the publications are American.

A glaring offence against seemly practice is described in *Physical Review Letters* (30, 1; 1973) by an editor of that distinguished journal, Professor S. A. Goudsmit. By that account, an article on laser-induced fusion was submitted to the journal and sent in the normal way to a knowledgeable referee. In due course, there emerged a referee's

report which must surely take the prize for the most unhelpful document of this kind—the referee by all accounts explained that all but two short paragraphs of his report could not be transmitted to the editors of the journal until they had been declassified. The author guesses that the classified parts of the referee's report refer to those parts of his own article which had to be omitted because they dealt with classified material.

This tale is not merely a description of a nonsense but a serious criticism of the way in which professional scientists conduct themselves. Is it really credible that serious professional people should write a scholarly opinion on a colleague's work and send this to the security men, not to the person for whom it is intended? Is it sensible, in any case, that the US Atomic Energy Commission should persist with a policy on classification which fails to distinguish between the kind of work where secrecy is legitimately applicable—where imminent practical applications are in question, for example—and the theoretical work or basic research typified by Dr Winterberg's article? To pursue a policy like this is to recreate the frenzy of the 1950s. And because, where laser-induced fusion is concerned, the Atomic Energy Commission has apparently been trying to prevent independent authors from publishing their entirely independent research, is it not time that the scientific community said what it thinks of policies like these?

Relativistic Forms

IN both quasars and pulsars some parts of a system almost certainly have speeds relative to other parts that are comparable with the speed of light, c . If so, then some parts have speeds of this order relative to any observer. Thus it becomes of practical significance to discuss the consequences of such motions for the observable features of a system.

As an academic exercise, many authors have considered the subject, and there exist frequently quoted papers, in particular by Penrose, by Terrell, and by Weisskopf; also there is a good account of certain aspects in a recent textbook by Meyer-Arendt (*Introduction to Classical and Modern Optics*, Prentice Hall, 1972). But now that the subject has gained more than academic interest, a new critical review by Mathews and Lakshmanan (*Nuovo Cim.*, 12B, 168; 1972) is specially timely, even though these authors mention topical applications only in passing.

Consider an observer O, in an inertial frame S, observing some given object K fixed in another inertial frame $\bar{S}$ which has uniform speed V relative to S, where V is comparable with c . If the applicability of special relativity is assumed, then what O sees at any instant is determined by

"relativistic" effects and by the different times taken by light to reach O from various parts of K.

Several familiar assertions, that presumably originated as rough shorthand descriptions of valid results, are false as they are often presented.

● "Bodies are seen to undergo the FitzGerald-Lorentz (F-L) contraction." In fact, this contraction becomes evident only after the observations have been reduced by a standard procedure allowing, in particular, for effects of different travel times for light. As such effects are of first order in c^{-1} , whereas the F-L contraction is a second order effect, in general they produce much greater apparent distortion in the object as actually seen. In other words, classical effects outweigh relativistic effects in determining apparent visual forms.

● "Linear motion of an object produces apparent rotation." Although this is valid for a sufficiently small rigid rod, it does not correctly describe the appearance of other moving objects.

● "A moving rigid sphere always appears spherical." In fact a fast-moving sphere must in general appear distorted in a complicated fashion, although it happens to be true that it always shows a circular profile.

These and other qualitative features are well clarified in the paper by Mathews and Lakshmanan. Quantitatively, their discussion prompts the following treatment (although it is not explicitly presented by them), which makes it possible to deal graphically with all possible cases.

First, consider any particular plane Π in $S, \bar{S}$ that contains the direction of relative motion; in the usual way let $Oxy, \bar{O}\bar{x}\bar{y}$ be rectangular frames in Π , fixed in $S, \bar{S}$, with axes $Ox, \bar{O}\bar{x}$ lying along each other and chosen so that $\bar{S}$

moves with speed V relative to S in the x direction. Write $\beta = V/c$, $\gamma = (1 - \beta^2)^{-1/2}$. Let t be time in S , and let $O, \bar{O}$ pass through coincidence at $t = 0$. Then a particle fixed in $\bar{S}$ at $P(\bar{x}, \bar{y})$ has a history in S expressed by the Lorentz formulae

$$x - Vt = \gamma^{-1} \bar{x}, \quad y = \bar{y} \quad (1)$$

Consider the view at $t = 0$ of the observer O stationed at the point O in S . He sees particle P at a point (x, y) of equations (1) such that a photon from $P(x, y)$ reaches O at $t = 0$. So P has $t = -r/c$, where $r = |OP|$, whence, from equations (1), $x + Vr/c = \gamma^{-1} \bar{x}$. If $\bar{x}$ is given, that is if P in $\bar{S}$ lies on a line of constant $\bar{x}$, it then follows that $P(x, y)$ in S lies on the locus (in polar coordinates r, θ)

$$l/r = \beta^{-1} \cos \theta + 1 \quad \text{where } l = \beta^{-1} \gamma^{-1} \bar{x} (\bar{x} > 0) \quad (2)$$

$$\text{or } l/r = -\beta^{-1} \cos \theta - 1 \quad \text{where } l = -\beta^{-1} \gamma^{-1} \bar{x} (\bar{x} < 0)$$

This is the set of branches convex towards the positive x direction of the family of similar hyperbolas having focus O , eccentricity β^{-1} , and vertices $(x((1 - \beta)/(1 + \beta))^{1/2}, 0)$ for $\bar{x} > 0$, $(x((1 + \beta)/(1 - \beta))^{1/2}, 0)$ for $\bar{x} < 0$. In S the lines of given $\bar{y}$ are $y = \bar{y}$. Thus the rectangular graticule in $\bar{S}$ is seen instantaneously from O in S as the mesh of hyperbolic branches (2), together with the lines of constant y . Thence the form of any given figure in the plane Π in $\bar{S}$ can be plotted in S as it is seen by O .

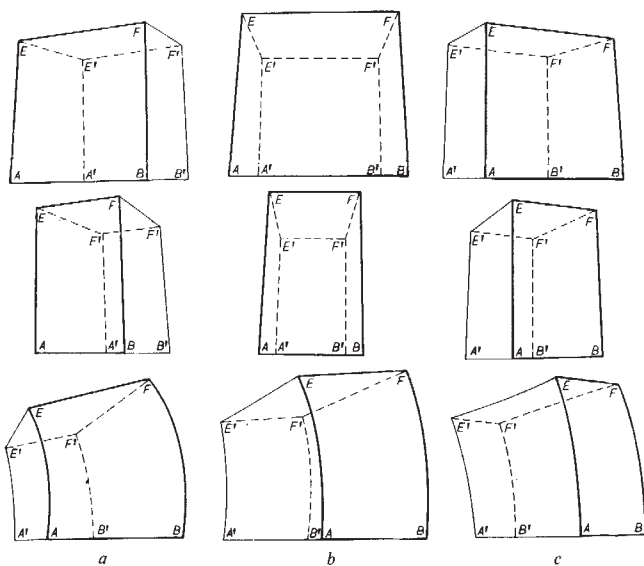
In particular, a rectangle in $\bar{S}$ with edges parallel to the axes is seen as having suffered contraction or expansion, combined with non-uniform shear, in the direction of motion. The apparent form of objects remote from the observer can, however, be obtained in general by using the asymptotes of the hyperbolic branches, instead of the curves themselves, and to this approximation the shear is, in general, uniform. This shows, as a special case, why a rod, at a large distance, appears to be rotated by the motion.

Similar results hold good in any other plane through Ox . So for three space dimensions the mesh can be completed by circles with axis Ox .

The procedure determines, in fact, the region in S that appears to be occupied by the object K when O views it at a particular instant. In order to describe precisely how O sees it, one ought then to apply the usual rules of perspective to the region. Mathews and Lakshmanan illustrate this in the case of a cubical box moving with one set of edges parallel to Ox with the results shown in their diagram, which is reproduced here.

Finally, consider again the particle P in (1) with $\bar{x} = 0$, $\bar{y} = 0$. At his time T , say, O sees P at position $x = Vt$ where $T - t = x/c$ which gives $x = VT(1 + V/c)^{-1}$. Thus $dx/dT = V(1 + V/c)^{-1}$. Now suppose that $V (= -W)$ is negative, then $-dx/dT = W(1 - W/c)^{-1}$. This is greater than W , for all $W > 0$, but it is also greater than c if $W > \frac{1}{2}c$. In this last case O may say he sees P approaching him in such a way that the distance to P seems to be decreasing at a speed greater than the speed of light. This is essentially the example of the phenomenon given by Mathews and Lakshmanan. It shows in an almost trivial way how an apparently natural measure of speed evaluated from observations might happen to give a value greater than c . It has also been suggested that the interpretation given to some observations of quasar phenomena may be a slightly more sophisticated instance of the same general type of occurrence. So long as the situation is understood, there is nothing "wrong" with it.

W. H. McC.



The bottom row shows perspective drawings of the visual shapes of a moving cube ($\beta = 0.8$) as seen: *a*, while approaching, the apparent position being at about 30° to the y axis; *b*, when the apparent position is closest to the observer; and *c*, while receding, at about 30° to the y axis. If the cube itself or its Lorentz contracted form were held stationary at the above apparent positions, it would appear as depicted in the top or middle row respectively. The objects in *a* and *c* would be further from the observer than in *b* and so should seem smaller in overall size, but this is compensated by choice of scale in drawing. Note the progressively decreasing length of the rear face (indicated by thick lines) as the cube moves past, and the fact that in *a*, even while the object is approaching, it is the rear face which is seen because of the apparent shear (and not the front face as in the case of the stationary objects). Reproduced from the paper by Mathews and Lakshmanan quoted in the text.

Infection in Pregnancy and Foetal Growth

INFECTION during pregnancy is well recognized as a significant cause of foetal loss and morbidity. Abortion, congenital anomalies, retardation of foetal growth, and leukaemia have been ascribed to a wide variety of organisms with varying degrees of suspicion or proof. The infections recognized as causing retardation of human foetal growth include rubella, cytomegalic inclusion disease and, less commonly, toxoplasmosis. Although the mother may show little evidence of illness the infective agents of these conditions all cross the placenta readily and infect the foetal tissues, particularly in the first half of pregnancy. Involvement of the developing central nervous system is frequent and may result in mental retardation, hydrocephalus or microcephaly. Bacterial infection can cause equally severe illness or death in the neonate, but bacterial infections in the mother have not been shown to cause retardation of foetal growth. In asymptomatic maternal bacilluria the babies tend to be of low birth weight as a result of premature onset of labour rather than failure of foetal growth (Wren, *Med. J. Aust.*, 2, 596; 1969).

R. L. Naeye has investigated the cellular basis of retardation of foetal growth seen in rubella and cytomegalic inclusion disease and has found that the basic defect is a deficiency in the cell populations of many of the organs throughout the body (Naeye and Blanc, *J. Amer. Med. Assoc.*, 194, 1277; 1965; Naeye, *Amer. J. Clin. Path.*, 47, 738; 1967). Confirmation that failure of growth may be a direct result of virus infection comes from the tissue culture work of Rawls and Melnick (*J. Exper. Med.*, 123, 795; 1966) who have shown that the doubling time for fibroblasts is prolonged from the normal of 24 hours up to 48 or 72 hours in cultures infected with rubella virus.

On page 460 of this issue of *Nature*, C. R. Coid and D. B. Ramsden, of the Clinical Research Centre, Harrow, report experiments on mice which show that an inapparent maternal infection with Cocksackie B3 virus on day 8 of pregnancy causes retardation of foetal growth. The ratio of serum albumin to α_1 foetoprotein was higher

in the newborn mice which were growth retarded than in control mice of the same gestational age although they matched those of immature control foetuses; this suggests an impairment in development of the plasma proteins. Further work on this model will clearly be needed to establish whether the viral infection causes retardation of foetal growth by an indirect effect, such as depression of maternal appetite, or through a direct action on the foetus such as that shown for rubella.

Although the enteroviruses have not been implicated as causing retardation of growth of the human foetus they are coming under increasing suspicion as causative agents for congenital anomalies. A large prospective study carried out by G. C. Brown at Ann Arbor has shown that inapparent maternal infections with Cocksackie viruses are significantly associated with a variety of congenital anomalies, particularly those affecting the heart. Sixty-five of 117 mothers giving birth to infants with cardiac anomalies had Cocksackie infections in pregnancy as compared with 68 of 219 controls. Most of the significant dif-

ferences between the groups was accounted for by infections with Cocksackie B3 and B4 viruses (Brown, *Arch. Environ. Health*, 21, 362; 1970). Indeed from the data of this survey it should be possible to confirm or refute the importance of maternal viral infection (or at least infection with enteroviruses, adenoviruses or influenza viruses) as a cause of retardation of human foetal growth. Conversely the experimental model produced by Coid and Ramsden could be useful for confirming the teratogenic potential of the Cocksackie viruses suggested by epidemiological surveys.

Additional support for a possible role of chronic infection in the causation of some cases of retardation of foetal growth is given by the finding of high levels of serum IgM in twelve of twenty-eight growth retarded infants (Yeung and Hobbs, *Lancet*, i, 1167; 1968).

Further work on the experimental aspects of viral infection in pregnancy may well show that a significant proportion of perinatal loss and morbidity is associated with inapparent viral infections in the mother.

J. S. W.

VIROLOGY

Pox in Monkeys

from our Medical Virology Correspondent

ALL nonhuman primates, as well as other animals, have a viral flora indigenous for that species and, although crossing of the species barrier undoubtedly occurs, it is not known to what extent this happens in nature. Sero-epidemiological data collected thus far do not support the hypothesis that a reservoir for smallpox exists in wild monkeys and apes, but natural poxvirus infections of nonhuman primates do occur.

Monkeypox virus was first identified in 1958 (P. von Magnus *et al.*, *Acta Pathol. Microbiol. Scand.*, 46, 156; 1959) during an outbreak of a vesicular eruptive disease among captive non-human primates in Copenhagen. The pustular disease was similar in distribution to variola in man and it was recognized as a poxvirus by its morphological appearance in the electron microscope and by its serological relationship to vaccinia. The first six cases of human

infection with monkeypox were identified 12 years later in West Africa. There was a prodromal illness of a few days' duration followed by a vesiculopustular rash which was peripheral in distribution. All six patients recovered, although three were severely ill. Monkeypox virus was isolated from four patients and serological tests were positive in all six patients. Two of the patients had occasionally eaten newly killed monkeys and others had played with the viscera of monkeys. Spread of the infection from person to person had not occurred and no evidence of infection was observed in households where patients with monkeypox lived.

In 1971, another virus (Chimp 9) was isolated from the kidneys of a chimpanzee which was illegally shot in the Republic of Zaire in an area where human cases of monkeypox had occurred (S. S. Marennikova and colleagues, *Bull. Wld Hlth Org.*, 46, 599; 1972). This virus was identical with the three "wild white" poxviruses isolated by R. Gispén of Utrecht in 1967 from the kidneys of apparently healthy *Cynomolgus* monkeys and these behaved more like smallpox than monkey-

pox. It is obviously essential that the extent of the relationship between all these virus strains be clearly established and a start in this direction has been made.

Marennikova and colleagues (*Arch. Ges. Virusforsch.*, **33**, 201; 1971) compared the morphology of five isolates of simianpox virus and found that four out of the five viruses formed a homologous group differing in some respects from other poxviruses. The other strain of virus, which was isolated from apparently healthy monkey kidney, was indistinguishable from variola virus. Rondle and Sayeed (*Bull. Wld Hlth Org.*, **46**, 577; 1972) studied the growth characteristics and serological reactions of three monkeypox virus strains and found that monkeypox strains constitute a homogeneous poxvirus entity but that they can be differentiated from other poxviruses and that serologically they are more closely related to variola than to vaccinia or cowpox viruses.

To date thirteen confirmed cases of monkeypox in man have been reported from Liberia, Sierra Leone, Nigeria, Zaire and the Ivory Coast and some possible implications should be considered. Is there in West Africa a smallpox-like disease in animals which can spread to human populations? It is of interest to note that a fatal pox disease in *Mycetes* and *Cebus* monkeys in remote Brazilian forests was reported by Blayer as long ago as 1922 (*Munch. Med. Wschr.*, **69**, 1009), yet an intriguing observation is that in spite of extensive field surveys monkeypox in monkeys seems to be rare in West Africa and even serological surveys have yielded poor results. Yet a corollary to the first question is what is the possibility of epidemics arising in man once the present primary protection against smallpox has waned? Many ecological factors are undoubtedly involved in the distribution of viruses and these have not been clearly defined; but the need for the continuation of smallpox surveillance programmes is paramount.

FOSSIL RECORD

Palaeontology Moves On

from a Correspondent

FOR the meeting of the Palaeontological Association at Oxford on December 18-20, the council invited talks on all aspects of the subject, which has evolved considerably in the past decade.

Six contributors were concerned with the origins of higher taxa; Dr J. W. Valentine (University of California, Davis) outlined more theoretical aspects of phyletic interrelationships, and Dr R. P. S. Jefferies (British Museum (Natural History)) discussed the origin of the chordates on the basis of the early Phanerozoic fossil record. Other

speakers based conclusions directly on fossil evidence, and in particular reflected the impact of scanning electron microscopic studies on skeletal micro-architecture and its phylogenetic significance.

The role of the palaeontologist in the interpretation of ancient environments received attention from several speakers, as did problems of functional morphology and palaeoecology of extinct organisms. All speakers in these categories revealed the extent to which palaeontologists have moved into the fields of ecology, and showed how ecological studies on living organisms are becoming an important factor in both the teaching of, and research in, the subject. Dr J. H. Lipps (University of California, Davis) reviewed work on adaptive strategies of Recent Foraminifera with its obvious consequences in the understanding of the palaeoecology of the group, and R. M. Kier (Smithsonian Museum, Washington) showed spectacular time lapse films of living echinoids which revealed many functional aspects; this work should prove valuable in assessing the nature of comparable features in related extinct forms.

Studies on regional faunal distributions and their role in the interpretation of ancient geographics received attention. Dr J. Murray (University of Bristol) outlined a Tertiary reconstruction of Western Europe based on comparisons with Recent foraminiferal

assemblages, and Dr M. Hart (Plymouth Polytechnic), who drew his evidence from land-based samples and also JOIDES cores, demonstrated the value of regional microfaunal studies in understanding the early history of the Atlantic.

HAEMOGLOBIN

Intermediates in View

from our Molecular Biology Correspondent

New essays in fitting mathematical models to haemoglobin oxygenation curves tend nowadays to provoke in the outsider, depending on temperament, either mounting hysteria or a contented indifference. Even to the interested, the dispiriting suspicion that one has been here before is hard to fight down. But this is deceptive, for much progress has been made, and in particular Perutz's model for the oxygenation mechanism, which seems to follow almost irresistibly from the structural differences between the oxygenated and unliganded forms, has placed something of an obligation on physical chemists to see how equilibrium and kinetic properties in solution fit into this quite explicit framework. An important step in this direction was made by Ogata and McConnell (*Biochemistry*, **11**, 4792; 1972) who, while adhering to the principles of a fully concerted mechanism as the basis of co-

States of Myosin

THE contraction of muscle involves a displacement of myosin cross-bridges between the thick and thin filaments. When ATP is absent the bridges are in contact with the actin filaments, while making an angle of about 45° with the myosin filament axis. In the presence of ATP, on the other hand, they lie perpendicular to the thick filaments. The movement of the bridges between these states is presumed to lead to displacement of the thick and thin filaments relative to each other.

Analysis of myosin ATPase kinetics has shown that the rate-limiting step is the dissociation of the products, ADP and phosphate, from the active sites. The dissociation of the actin-myosin complex by the ATP is rapid. Thus when ATP is present one observes only the state in which the myosin is complexed with the hydrolysis products. An article by Mannherz *et al.* in next Wednesday's *Nature New Biology* (February 21) deals with the situation brought about by use of an ATP analogue, α,β -methylene-ATP, which differs from ATP in that the release of the hydrolytic products is rapid. Thus in the presence of this form the predominant structural state will be the

one in which the active sites carry substrate rather than product.

The results show that the actomyosin complex in this system is unstable, myosin being dissociated from F-actin in the ultracentrifuge. In muscle fibres this effect is reflected by complete relaxation on addition of the analogue. Furthermore, the characteristic 145 Å reflexion in the X-ray pattern of insect flight muscle containing ATP vanishes when the ATP is replaced by the analogue. Because the 145 Å reflexion is associated with the perpendicular position of the cross-bridges, and its absence is also a characteristic of muscles in rigor, it follows that the bridges either enter their angled configuration, or possibly are in some way randomized.

These results support the view that the ATP complex is associated with a different structure from that of the ADP complex, and that the ATP is normally hydrolysed on the back-stroke of the bridges before they return to their perpendicular orientation. This sequence is also required by the kinetic rates, which indicate that the enzymatic activity resides in myosin uncomplexed with actin.

operativity—that is to say a two-state conformational equilibrium, involving low and high-affinity (R and T) forms—have expanded the model by permitting differences between the intrinsic oxygen affinities of the α and β -chain haems. The further assumption that there is no cooperativity between the ligand binding steps in either state, so long as there is no transition from one to the other, forbids any sequential component in the mechanism.

The choice of numerical parameters to test the model against experimental data is never, of course, wholly self-evident, and admits of a certain degree of sophistry. Ogata and McConnell base their choice of oxygen dissociation constants for the α and β chains in the R state on values reported for single chains (concerning which, it must be conceded, there has been some argument). Those for the T state they draw from their own measurements on hybrids, in which either the α or the β chains are in the cyanmet-form, and therefore functionally silent. The only other parameter of the model is the allosteric constant, L , which governs the position of the equilibrium between the T and R conformational states, with all haems unliganded. The effective value of L is modified by the presence of the cofactor, diphosphoglycerate, or of an analogue, because this binds to the T state only and so shifts the equilibrium in its favour by a factor that depends on the cofactor concentration and its (known) binding constant.

Ogata and McConnell use a spin-labelled cofactor analogue, the paramagnetic resonance spectrum of which is sensitive to its precise environment, and indicates that the two hybrid species, with the α and β chains respectively in the cyanmet-form, are conformationally indistinguishable, at least by this criterion. With the aid of a value for the parameter L , which is set at 3,000, the system at any given cofactor concentration can be completely described in terms of the balance of T and R forms, and the degree of saturation of the α and β chains in either state. At any given level of oxygen saturation, it turns out that the fractions of molecules in the R state are always highly, and those in the T state slightly, saturated, that is to say that the unliganded and fully liganded forms predominate in accordance with the observed cooperativity. In the absence of cofactor, the T $\rightarrow$ R transition occurs largely when the total ligand saturation is about half, whereas in its presence, most of the transition ensues only when three oxygen molecules have been bound. This agrees with indications from several experimental lines. Saturation curves of two abnormal haemoglobins, one an α , the other a β -chain variant, can be fitted if the binding constants in the T

states of the abnormal chain, and in particular the values of L , are grossly changed, so as to make the R form accessible in all binding states.

Another β -chain variant, haemoglobin Kansas, where the replacement occurs at the $\alpha_1\beta_2$ subunit interface, and so might well be expected to affect the relative energies of the two conformational states, shows the same kind of anomaly in reverse by two very direct criteria. Hopfield, Ogawa and Shulman (*Biochem. Biophys. Res. Commun.*, **49**, 1480; 1972) have found that the rate constant for carbon monoxide uptake is independent of the degree of saturation, whereas in normal haemoglobin there is an increase in rate, in consequence of the appearance of the rapidly reacting R form, at a saturation corresponding to nearly three ligand molecules bound. Moreover, Ogawa, Mayer and Shulman (*ibid.*, 1485) find that oxyhaemoglobin and carboxyhaemoglobin Kansas are pulled into the R form by diphosphoglycerate or its analogues, as reflected

by the appearance of features in positions of the proton magnetic resonance spectrum uniquely associated with the deoxygenated condition of the normal protein. This means that, even when oxygenated, the T state is energetically sufficiently accessible to be populated with the aid of the additional stabilization conferred by cofactor binding.

Supporting the many lines of evidence that now point to higher oxygen affinity of the α compared with β chains in the T state are experiments of Huestis and Raftery (*ibid.*, 1358), who have introduced a trifluoroacetyl group on cys-93 of the β chain. The ^{19}F NMR spectrum emanating from this group is very sensitive to events involving the β chain. Comparison of the oxygenation-induced shift with spectrophotometric measurements of total oxygen uptake confirms that oxygenation must start at the α chains. Two minuscule dimples in the ^{19}F NMR spectrum at intermediate stages of oxygenation are interpreted in terms of signifi-

B Cells Responsive to Phytohaemagglutinin

Not so long ago the thought that lymphocytes could actively proliferate was far from the minds of pathologists and haematologists. When Nowell concluded in 1960 that phytohaemagglutinin (PHA), a protein extracted from seeds of *Phaseolus vulgaris*, caused active mitosis of human peripheral blood lymphocytes *in vitro* there was much amazement. Cytogeneticists were the first to take advantage of the result in order to display human chromosomes. Slowly immunologists came to realize that their favourite cell could perform an interesting trick *in vitro*. Concomitant with this realization came the knowledge that lymphocytes were heterogeneous in relation to their anatomical origins. In 1968 several investigators, working largely with mice, concluded that cells of thymic origin (T cells), rather than those derived from the bursa or its equivalent in mammals, were the PHA-responsive cells.

With great alacrity this finding was extrapolated to the human species and it was found that in immunodeficiency disease syndromes, which were supposed to involve T-cell insufficiency, PHA-responsive lymphocytes were few and far between. The advantages of having an *in vitro* measure of T cells in man were enormous and there seemed little reason to suppose that what had been proven beyond doubt in the mouse did not also apply to man. In next Wednesday's *Nature New Biology* (February 21) Phillips and Roitt present evidence which could prick this bubble of enthusiasm. Specifically the authors suggest that both B and T cells can respond to PHA in man in quite con-

ventional cultural conditions.

Phillips and Roitt first found that approximately 30 per cent of blast cells in PHA cultures of peripheral blood lymphocytes stain with a fluorescent anti-light chain antiserum. This result suggested either that T cells develop immunoglobulin (Ig) on their surfaces when responding to PHA or that B cells, which are widely thought to have immunoglobulin on their surfaces, could be included among the responsive cells.

When tonsillar lymphocytes were run down columns coated with anti-Ig, the resulting T cell-rich population was transformed with PHA *in vitro*, but as few as 5 per cent of staining cells could be counted. By using dextranase it was possible to recover the retained B cells from the column coated with anti-Ig and these gave rise to 86 per cent staining blasts when cultured with PHA. Incidentally no controls are quoted in which dextranase was used to treat T cells to see if they then developed immunoglobulin on their surfaces. But aside from this peccadillo the evidence of Phillips and Roitt strongly suggests that B cells in man can transform to PHA and that PHA transformation is thus an unreliable criterion of T-cell numbers in the human species.

It seems that care must be taken in attributing any characteristic as an absolute marker of either T or B cells and that extrapolation between species in relation to the distinguishing features of the two cells is hazardous. It should be noted that this argument applies to the use of surface immunoglobulin as a criterion of the B cell.

cant concentrations of partially oxygenated species, which are inferred to be in some wise different from the pure T and R states. Taken at face value this would seem to add a new degree of complexity in the system.

This is perhaps a good moment to relate that my account some time ago (*Nature*, 237, 481; 1972) of the work of Perutz and his colleagues on the structural and functional properties of ferrous-ferric hybrid haemoglobins occasioned a frightful eruption, and a rain of shrapnel. An irate reader wrote to state that Perutz had made a rudimentary error in his assignments of high and low-spin spectroscopic features in the visible, and had in other respects abused his data, and I was charged with perpetuating if not amplifying his errors. Having taken it all on the chin, I will not tax readers with the details: the model as outlined is now hanging fire, while Perutz prepares new arguments, which are promised in detail soon.

POPULATION CYCLES

Why Study Dispersal?

from a Correspondent

POPULATION cycles of abundance of small rodents have kindled interest in ecologists for almost half a century, and several theories purporting to explain them have been put forward. All these theories have been rather less than convincing because they lacked reinforcement from experiments in the field. Some interesting observations on rodent cycles are now put forward by Krebs, Gaines, Keller, Myers and Tamarin (*Science*, 179, 35; 1973).

Krebs *et al.* started with the ideas put forward by D. Chitty (*Proc. Ecol. Soc. Aust.*, 2, 51; 1967) that the genetic composition of the population changes during a cycle in numbers and that internecine strife is the factor ultimately responsible for population decrease. During a five-year period (1965 to 1970) Krebs and his colleagues studied the anatomy of field vole populations (*Microtus pennsylvanicus* and *M. ochrogaster*) in southern Indiana. The mechanics of the decline were obvious—a reduction in survival of early juveniles and in birth rate, but more important an increased mortality among the older animals. This increased mortality affected males and females differently at different times of the year. That mortality acts selectively in this way is thought to demonstrate that extrinsic factors are not the chief forces in population decline.

In order to investigate the intrinsic quality of population decline, Krebs *et al.* built a *Microtus*-proof enclosure and compared population increase within the fence with a control area outside it.

The number of animals in both groups increased sharply but became excessively high only within the enclosure. It seemed that prevention of dispersal was largely the root cause. In the adjacent control area, the percentage losses of voles as a result of dispersal were much higher during population increase than during peak densities of population decline.

Using the genes *Tf* (transferrin) and *LAP* (leucine aminopeptidase) as markers, the authors found at these two loci large changes in gene frequencies which could be related to dynamic change in population number. During the increase phase, 89 per cent of the losses of heterozygous females (Tf^e/Tf^c —*c* and *e* are alleles of the *Tf* gene) from the control populations were caused by dispersal. This suggests that demographic events are genetically selective and dispersal losses are not equal over all genotypes. This is the first time a genotype for dispersal has been demonstrated in a natural population.

The hypotheses of population regulation by social stress have to explain the finding of *Microtus* populations in the enclosures at densities many times higher than normal without "stress" taking an obvious toll. Prevention of dispersal changes the quality of the enclosed populations compared with that of the controls. In spite of the high density they continue to breed, but the behavioural effects of crowding do not seem to cause voles to die. Only when starvation takes effect by decreasing survival and reproduction does the rate of increase start to drop off. However productive they may be, fencing experiments turn up many new questions. For

example, does the differential dispersal rate of animals during the increase phase of the cycle cause the quality of voles remaining at peak densities in the wild to be different from the quality observed at far higher densities in enclosures?

Several experiments are suggested by Krebs *et al.* to examine the components and influences of dispersal. The first is to attempt to cause abnormal densities in other rodent species by preventing dispersal. Second, one-way doors fitted to fenced enclosures should allow population regulation through dispersal to occur. Third, because dispersal is relatively unimportant once peak density has been achieved, closing the doors at this time should have no effect on normal decline. Krebs *et al.* themselves state that they do not know whether the genetic changes they describe are causally related to population changes, or are just side effects of other changes.

The ideas reported here naturally refer to existent populations of voles. Dispersers from increasing populations colonize unoccupied areas and, because of the increased incidence of heterozygotes among their number, will establish populations rich in *Tf* heterozygotes. This does not seem to bestow any observable ecological fitness at this stage in the demographic pattern. Evidence from island studies indicates that isolated rodent populations are much like land-locked populations in their demographic development. In the light of the new observations it is these populations founded by colonizers from other populations, and in which dispersal is largely prevented, that should now be closely scrutinized.

Cyclic AMP and Chloramphenicol Acetyl Transferase

THE antibiotic inactivating enzyme chloramphenicol acetyl transferase is one of the numerous enzymes in *Escherichia coli* the synthesis of which seems to be regulated by cyclic AMP, but unlike the inducible enzymes involved in the metabolism of sugars, which are also regulated by cyclic AMP, chloramphenicol acetyl transferase is made constitutively. How cyclic AMP regulates the synthesis of chloramphenicol acetyl transferase is the question which de Crombrughe, Paston, Shaw and Rosner have been investigating. As they report in *Nature New Biology* next week (February 21) it seems that both cyclic AMP and the nucleoside tetraphosphate ppGpp independently stimulate the synthesis of this enzyme.

The group used an ingenious and very sensitive assay for chloramphenicol acetyl transferase and found that cyclic AMP stimulates its synthesis about twofold. This indicates that the synthesis of this enzyme is not as strictly con-

trolled by cyclic AMP as is the synthesis of β -galactosidase. To investigate the control mechanism further, de Crombrughe *et al.* set up a cell-free system which synthesizes chloramphenicol acetyl transferase and responds to cyclic AMP which then stimulates a fifteenfold increase in the synthesis of this enzyme. This stimulation is specific, but ppGpp in the absence of cyclic AMP also enhances synthesis of the enzyme. By contrast, ppGpp alone does not stimulate synthesis of enzymes of the *lac* operon and other inducible enzymes. In short, the synthesis of chloramphenicol acetyl transferase is enhanced by cyclic AMP and ppGpp alone, whereas synthesis of other *E. coli* enzymes by ppGpp depends on the presence of cyclic AMP.

These differences justify further experiments aimed at elucidating in detail the mechanism of regulation of synthesis of this antibiotic inactivating enzyme.

ENZYMES

RNase H Activity

from our Cell Biology Correspondent

WHEN an enzyme becomes as fashionable as reverse transcriptase from RNA tumour viruses has become since its discovery in 1970 one can guarantee that any interesting new claim about its properties will rapidly be confirmed or refuted, as the case may be. That is perhaps the chief benefit stemming from such competitive situations. Take, for example, the seminal observation reported by Mölling and her colleagues in 1971 (*Nature New Biology*, **234**, 240). They found that reverse transcriptase isolated from virions of avian myeloblastosis virus (AMV) has an associated ribonuclease H activity which specifically digests the RNA moiety of an RNA-DNA hybrid molecule but which does not digest single stranded RNA or double stranded RNA or DNA. This report stimulated several groups to pursue further the characterization of this RNase H activity, and during the past couple of months the fruits of their labours have appeared in print.

Baltimore and Smoler (*J. Biol. Chem.*, **247**, 7282; 1972) have reported that the DNA polymerase and RNase H activities of reverse transcriptase from AMV cannot be separated by analytical chromatography or centrifugation. And by using tailor made synthetic RNA-DNA substrates they established that the RNase H activity releases oligonucleotides and mononucleotides with 3' hydroxy groups and 5' phosphoryl groups. Because some of the products of digestion are oligonucleotides they describe this activity as an endonuclease.

Keller and Crouch (*Proc. US Nat. Acad. Sci.*, **69**, 3360; 1972), who also found that the DNA polymerase and RNase H activities of reverse transcriptase cannot be separated, studied the degradation of both synthetic RNA-DNA molecules and the RNA containing DNA of the plasmid Col E₁. Like Baltimore and Smoler they find that the products of digestion are oligonucleotides as well as mononucleotides, but their data also indicate that the RNase H activity requires a substrate, the RNA moiety of which has free ends. This would indicate that the viral RNase H activity is an exonuclease but it digests internal phosphodiester bonds presumably by leapfrogging from the free end to some internal position in the RNA chain. Whether such an enzyme should be called an endonuclease or an exonuclease is almost an argument over semantics. Keller and Crouch also established that the RNase H activity of AMV particles is distinct from the RNase H activity in chick cells and KB cells, an important point when it comes to deciding the origin of the enzyme.

Grandgenet, Gerard and Green (*J. Virol.*, **10**, 1136; 1972) have, by screening ten different viral reverse transcriptases, established that an RNase H activity is a universal property of reverse transcriptases from RNA tumour viruses. They have also shown that enzymes from different viruses have slightly different specificities for synthetic substrates, for example only the reverse transcriptases from Moloney mouse sarcoma leukaemia viruses and RD feline leukaemia viruses hydrolyse the poly(U) of poly(U).poly(dA).

A more interesting observation reported by this group (*Proc. US Nat. Acad. Sci.*, **70**, 230; 1973) is that the reverse transcriptase of AMV occurs in two separable forms: one consists of two polypeptide chains, an α chain with a molecular weight of about 65,000 and a β chain with a molecular weight of about 105,000; the other form is a single polypeptide chain with a molecular weight of 65,000. The two forms of the enzyme have antigenic determinants in common, the same template specificity and both RNase H and DNA polymerase activities. Reverse transcriptase from viruses other than avian RNA tumour viruses resembles the second form of the AMV enzyme, and consists of a single polypeptide chain. These data coupled with those of Keller and Crouch, and the properties of various mutants of avian RNA tumour viruses, strongly suggest that the α chain is coded by the viral genome. But what

is the origin and function of the β chain? Perhaps this is the beginning of a story that will prove to be as complex as that of the origin and mechanism of action of phage Q β RNA replicase.

The question of the role of the RNase H activity of viral reverse transcriptases is, of course, a matter of considerable speculation. This activity may well be necessary for the synthesis of a double stranded DNA provirus from a single stranded RNA viral genome. But that remains to be proven.

BIOLOGICAL CONTROL

Attack on Thistles

from our Plant Ecology Correspondent

IN spite of the publicity which has been evoked by the successful biological control of certain species of pest, these success stories are still few in number. This is especially true of the control of plant pests by the introduction or encouragement of herbivore populations. Classic examples are the control of *Opuntia inermis* in Australia by the beetle *Cactoblastis cactorum*, and that of *Hypericum perforatum* in the rangelands of North America by the beetle *Chrysolina quadrigemina*.

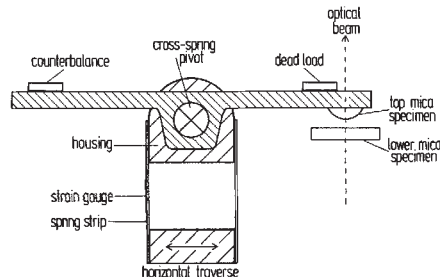
It may seem strange that in north-west Europe, one of the most intensively farmed areas in the world, there are no examples of effective control of a weed species by introducing a predator. This is not because the area is lacking

Shear Properties of Molecular Films

AN investigation of shear in molecular films, which has a bearing on the boundary lubricating action of long chain amphipathic molecules (those with one end hydrophilic and the other end hydrophobic), is described by Israelachvili and Tabor in next Monday's *Nature Physical Science* (February 19). Previous related experiments have been carried out by Bailey and Courtney Pratt (*Proc. Roy. Soc.*, **A227**, 500; 1965) who studied calcium stearate films on curved mica sheets. These experiments used low pressures, and the sheets were flexible; experiments using metal surfaces have also been carried out, but in these the area of molecular contact was not known precisely. In the experiments reported by Israelachvili and

Tabor, both thickness and area of sheared film were measured and contact pressure could be varied from 10^6 to 10^7 N m⁻².

The experimental arrangement is shown in the figure. The mica sheets, with backs silvered, are glued to cylindrical glass supports and viewed by multiple-beam interferometry to determine the area of contact. The load applied (dead load) can be up to 50 g, and the sliding speed can be varied between 3 and 40 μ m s⁻¹. Mono and multimolecular stearate films have been investigated. The results show that the first adsorbed monolayer is firmly attached to the mica substrate, and the shear strength (3 to 4×10^6 N m⁻²) is much the same as that reported by Bailey and Courtney Pratt, and others. The thickness of the monolayer, 48 ± 3 Å, suggests that molecules stand normal to the mica surfaces. At contact pressures of up to 10^7 N m⁻², the limit possible with the present experiment, the molecules of the monolayer remain oriented normal to the surface of the mica; thicker molecular layers are, however, easily dislodged and broken up by the sliding process.



in weed species, nor is it attributable to lack of effort in attempting to find suitable biological control agents. For example, the creeping thistle (*Cirsium arvense*) is a troublesome weed of pastures in Britain and many other countries, both in Europe and North America. Following the work of Zwölfer on the insect predators of this weed, a chrysomelid beetle, *Haltica carduorum*, which is a native of southern Europe, was introduced into two British localities in 1969 (Claridge *et al.*, *Entomologist*, **103**, 210; 1970).

The outcome of these introductions is now described by C. R. B. Baker, R. L. Blackman and M. F. Claridge (*J. Appl. Ecol.*, **9**, 819; 1972). Adult beetles imported from France were originally released in June 1969 at Silwood Park, Berkshire, and Llantwit Major, Glamorgan. They bred during the summer, but few survived the 1969–1970 winter. Those which did survive were first seen in mid-April, when the thistles had just begun regrowth after winter dormancy. Further introductions were made in summer 1970, but once again survival was poor.

Detailed studies of these populations showed that egg production was low and mortality was high at all stages in the life history of the beetle. Laboratory studies were carried out on the environmental requirements of the insect and it was found that there was a strong positive correlation between the rate of egg production by each female and temperature over the range 14° to 22° C. Egg mortality in the field was around 30 per cent when day temperatures were more than 20° C, but rose to 100 per cent if 15° C was not exceeded. Mortality among larvae was also high at low temperatures. Winter survival of adults was not studied in the laboratory.

The geographical distribution of *Haltica carduorum* in southern Europe coincides with situations where day temperatures in excess of 20° C can be expected regularly during the summer. Evidently the British climate is not so adverse that the completion of the life cycle is totally inhibited in this species, but it does seem to be detrimental to the maintenance of a viable population. Mean temperatures of 13° to 17° C, which were recorded in the field during these trials, are evidently sub-optimal for several stages in the life history of *Haltica*.

An effective biological control agent is one which is specific in its predation, which significantly reduces its prey population and which can maintain viable populations under the prevailing climatic conditions. Evidently *Haltica carduorum* fails on the third count; its effectiveness on the second count is also open to question. There are predators of *Cirsium arvense* with more

northerly distributions and which are absent from Britain for reasons other than climatic ones, such as isolation by the post-glacial eustatic rise in sea level which has severed Britain from the Continent. The search for an efficient biological control agent of the creeping thistle must now centre upon these species.

GAS DISCHARGES

Computing Streamers

GALLIMBERTI reports in a recent issue of *Journal of Physics D* (**5**, 2179; 1972) that he has constructed a quantitatively successful model of the propagation of a streamer discharge in a gas which makes use of a simple energy balance criterion. In the past the predictions of streamer models have not been particularly accurate.

A streamer is a weakly ionized and slightly luminous channel which starts from the positive electrode and has at its tip a high concentration of positive charge. The streamer grows as shown in the diagram—photoelectrons produced in front of the tip by photons each generate their own small ionization avalanches by virtue of the local field around the streamer tip. In air at

atmospheric pressure, for example, a field of more than 26 kV cm⁻¹ is necessary for avalanche development to be viable.

Unfortunately the equations governing the development of a series of avalanches are, in practice, impossible to solve numerically; Gallimberti makes the simplifying assumption that the avalanches in front of the streamer tip can be represented by a single avalanche which starts immediately ahead of the streamer, somewhere along the line A but within the active region. The problem is then to calculate where the equivalent avalanche starts. Gallimberti uses as the basis of this calculation the energy balance equation

$$W_g + W_{\text{pot}} = W_1$$

where the parameters are respectively the gain of energy as a consequence of the applied field, the difference between the potential energies of the streamer tip and the sphere of positive ions created during the development of the equivalent avalanche, and the total loss of energy during the formation of the new avalanche.

During the computing process the lengthening of the streamer caused by each successive equivalent avalanche was reckoned to be twice the radius of

Orbiting Systems, Mass Loss, and Gravity Pulses

THEORETICAL physicists continue to be intrigued by the problem of how large quantities of gravitational radiation might be produced in plausible astronomical situations. In next Monday's *Nature Physical Science* (February 19), Jackson and Matzner describe the effects of sudden loss of mass from a primary object on a hypothetical small companion in orbit around the primary. Because the mass of the primary (M) changes abruptly, the secondary (mass m) experiences a changing acceleration. This "jerk", defined as the third time derivative of position, or rate of change of acceleration, can become very large for large M . Such a situation provides a powerful, if short lived, mechanism for the production of gravitational radiation, because the third derivative of the quadrupole moment of the pair m, M becomes large, and the rate of production of quadrupole gravitational radiation depends on the square of this derivative.

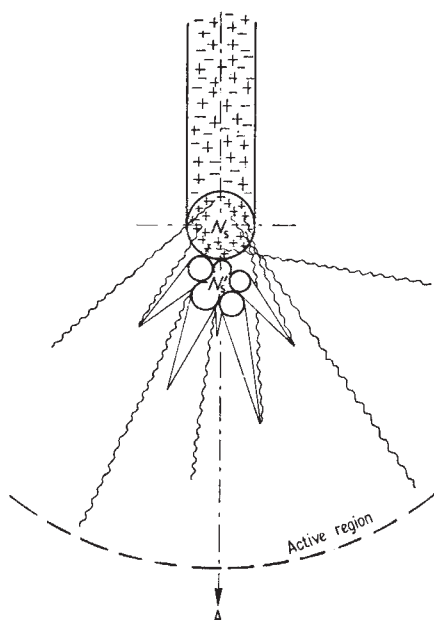
Some curious features emerge from the details of the analysis which Jackson and Matzner have made. If $\dot{M}$ is sufficiently large, the gravitational radiation shows similarities to radiation from a particle in a circular orbit; but in the case of the jerk mechanism information about the central gravity field—that is, about the nature of the mass M —is contained in the field equations. This seems to be a result of the requirement

that the speed of light is constant; the information that M is changing must be transmitted throughout M , and this will take a different time for diffuse objects, compact stars, or black holes.

Could this jerk mechanism have a bearing on the seemingly large flux of gravitational radiation reported by Weber to be coming from the galactic centre? If the loss of mass occurs at the galactic centre, from a compressed object, and if the Sun is taken as the secondary particle, then the flux rate observed at the Earth should be only 10⁻⁷ times that claimed by Weber. "It is tempting," say Jackson and Matzner, "to attempt to sum the contributions of many particles m surrounding a central body undergoing mass loss." This would only help with the observational problem, however, if the jerk radiation originates at the small particles and in some sense in phase; then a reverberation could sweep the cloud of small particles as the information about the loss of mass from M spreads outwards.

Because the work described in next Monday's *Nature Physical Science* is based on the weak field approximation, the intriguing speculations encouraged by this last possibility should really hang fire until similar calculations have been carried out for strong fields. Jackson and Matzner promise that such calculations are now under way.

the tip of the avalanche. A streamer ceases to propagate, of course, when the local field in the vicinity of the tip of



The way in which a streamer grows. New ionization phenomena can only take place in the active region. (From *J. Phys. D.*, 5, 2183; 1972.)

the streamer is no longer sufficient to allow further avalanches to form. Galimberti's programs yield the length and velocity of the streamer, the current and charge in the external circuit, and the charge in the streamer tip, all as functions of time and for a given arrangement of electrodes.

The accuracy of the calculations can be judged by a comparison between calculation and experiment for an electrode arrangement of a square rod separated from a plane by 150 cm. The applied voltage at the start of streamer formation was 125 kV in this case. It turned out that the computed and measured values for the total streamer lengths were 10.9 and 11.2 cm, and the initial velocities 0.23 and 0.28 cm ns⁻¹.

MICROTEKTITES

Bottle Green Variety

from our Geomagnetism Correspondent
TEKTITES, those strange natural glass bodies with diameters usually of several cm, are apparently limited to four large areas (strewnfields) and four corresponding ages. The Australasian, Ivory Coast, Czechoslovakian and North American strewnfields have approximate ages of 0.7, 1.0, 15.0 and 35.0 million yr, respectively—ages which in some cases are supported by more than one method of dating. The Australasian tektites, for example, have been dated by both K-Ar and fission track methods and are found in a layer coinciding with the Brunhes-

Matuyama geomagnetic reversal boundary. Microtektites (diameters <1 mm) are also associated with the two most recent strewnfields, having been found in adjacent deep sea sediment cores. Most of these microtektites ("normal microtektites") are physically and chemically similar to the larger tektites, but a few (0 to 20 per cent in any given core) have significantly different properties. These are the so-called "bottle green microtektites"; and the fact that they differ from normal microtektites has led several workers, beginning with Martin (at the US Meteorological Society Conference in 1967), to suggest that they are igneous glasses rather than proper tektites.

Martin has been followed in this by several others; but Glass (*J. Geophys. Res.*, 77, 7057; 1972) has now put paid to the whole idea by showing that although bottle green microtektites differ in some (but not all) respects from the normal variety they differ even

more from igneous glasses. For one thing, a search of more than seventy deep sea cores from around the world has shown that bottle green microtektites are found only in cores which contain normal microtektites and, moreover, only in the particular sediment layers containing the normal bodies. On the other hand, the converse is not true—all normal microtektites are not associated with bottle green ones. Thus bottle green microtektites were found in both cores containing Ivory Coast microtektites but in only three of the thirteen corresponding Australasian cores. Even those cores containing normal microtektites but no bottle green microtektites, however, contain yellow-green varieties with physical and chemical properties intermediate between the two. In this sense, the bottle green-normal association is enhanced, and is made even stronger by the coincident ages (because of coincident layering).

Palaeomagnetism and *G. truncatulinoides*

IN next Monday's *Nature Physical Science* (February 19), Theyer shows how palaeomagnetism has been used to invalidate what was once one of palaeontology's most reliable datum planes. The plane in question is defined by the first appearance of the planktonic foraminifer *Globorotalia truncatulinoides* and was taken to mark the onset of the Pleistocene at about 1.8 million yr ago. But if this interpretation were correct, the appearance of *G. truncatulinoides* should lie within the Matuyama reversed geomagnetic polarity epoch, whereas Theyer finds that in the southern hemisphere, south of 36° S, it lies within the earlier Gauss normal epoch.

Theyer's evidence comes from twelve palaeomagnetically dated and six other deep-sea cores from south of Australia and the Tasman Basin. Because radiolarians are the most widely used biostratigraphic correlators with the geomagnetic polarity-time scale, Theyer has used them in conjunction with already available palaeomagnetic measurements to match the cores to the time scale. Having done this, he finds that in ten of the palaeomagnetically dated cores *G. truncatulinoides* either first appears close to the base of the Gauss epoch or is already present towards the end of the epoch. Moreover, it is not possible to attribute the presence of *G. truncatulinoides* in this time period simply to the effects of reworking or contamination, for the populations are strong and, in those cores deposited far enough north to have experienced climatic variations, the abundance of *G. truncatulinoides*

fluctuates in phase. Correlations with other planktonic foraminifers also tell the same story.

One of the implications of the appearance of *G. truncatulinoides* some 1.0 to 1.5 million yr before its previously known appearance in lower latitudes is that the supposed evolution of *G. tosaensis* into *G. truncatulinoides* at the Pliocene-Pleistocene boundary cannot be correct. Theyer believes, therefore, that the two are subspecies or even ecological variants which appeared in different areas at roughly the same time. The evidence is that *G. tosaensis* is not found at all in cores from south of 36° S and that others have already reported the appearance of *G. tosaensis* at the base of the Mammoth polarity event in a tropical core.

To put it another way, the suggestion now is that *G. truncatulinoides* and *G. tosaensis* appeared more or less simultaneously, the former at latitudes higher than 36° S and the latter at lower latitudes but probably with a very slight overlap. The latitude separation, in turn, implies environmental (probably climatic) control on the evolution into the two subspecies.

Finally, Theyer's revision has an interesting general consequence. Because the appearance of *G. truncatulinoides* has been regarded as one of the most reliable Neogene foraminiferal datum planes, it has been used in numerous studies involving latitudes higher than 36° S. Presumably, therefore, if Theyer is right, the conclusions drawn in previous studies are wrong and also require revision.

This evidence, although strong, is circumstantial; but more direct support comes from comparisons of physical and chemical characteristics. The Australasian and Ivory Coast bottle green microtektites are very similar in appearance; they are transparent pale green and vary in shape from spherical to irregular. But by contrast with the range of smooth to grooved and pitted surfaces of the normal microtektites, the bottle green ones have deeply corroded surfaces. Thus the normal and bottle green microtektites are quite distinguishable from each other on the basis of surface texture and colour (although more easily in the case of the Australasian bodies). On the other hand, in both areas the normal and bottle green microtektites are similar in that they do not contain crystalline inclusions—and this distinguishes them from igneous glasses. Moreover, although the bottle green microtektites differ from the normal variety in having no vesicles, they also differ in this respect from igneous glasses. Finally on the physical side, bottle green microtektites are found to have higher densities and refractive indices than most normal microtektites; but the similarities are maintained in so far as the group of high-magnesium Australasian tektites reported by Chapman and Scheiber (*J. Geophys. Res.*, **74**, 6737; 1969) also have the higher densities. In short, the density ranges for normal and bottle green microtektites overlap.

As far as chemistry is concerned, the Australasian bottle green microtektites have significantly different compositions and trends from those of the normal microtektites. The two Ivory Coast types have different compositions—the bottle green ones have lower SiO_2 , Na_2O and K_2O , but higher MgO , FeO and CaO —but the same oxide trends. Moreover, the bottle green microtektites from the two areas have similar compositions. The normal Ivory Coast microtektites are, however, in many ways similar in composition to high silica Australasian bottle green microtektites, the chief difference being the lower FeO and the higher CaO and TiO_2 of the latter. And the bottle green Australasian microtektites overlap in composition with the high magnesium tektites of Chapman and Scheiber.

All this is very confusing; but what it amounts to is that the normal and bottle green microtektites have both chemical differences and similarities. The fact is, however, that whatever the differences are, they are distinct from the differences between microtektites and igneous glasses. This is particularly apparent when compositions are plotted on an $\text{MgO-K}_2\text{O-Na}_2\text{O}$ ternary diagram. Both tektites and microtektites have higher MgO and lower Na_2O

COSMOGENY

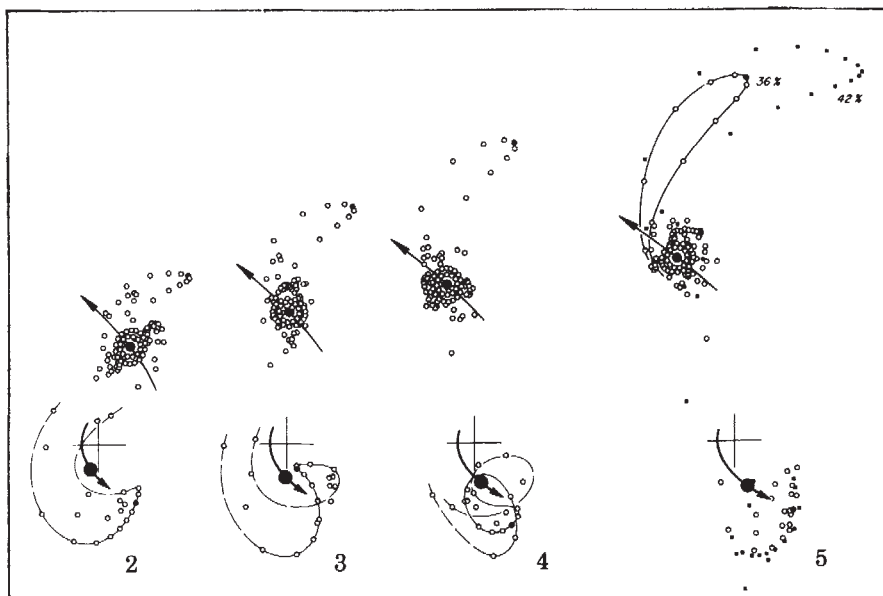
Origin of Bridges and Tails in Galaxies

by our Cosmology Correspondent

THE bridges and tails seen in some galaxies may simply be tidal relics of close encounters, according to Toomre and Toomre (*Astrophys. J.*, **178**, 623; 1972). They have studied such events in a "deliberately simple minded" computer simulation in which each encounter involves only two galaxies and is roughly parabolic, and in which each galaxy is idealized as a disk of non-interacting test particles initially orbiting a central mass point.

Two particularly interesting features emerge from the analysis. After a

small companion passes such a galaxy, the primary disk may deform into an arm or a bridge extending towards the secondary, together with a counter arm on the opposite side. After a similar encounter with an equal or more massive partner there is a tail on the far side of the primary; on the near side there is an "avalanche" of particles which are mostly captured by the satellite galaxy (see diagram). Toomre and Toomre also offer specific models to account for the shapes of the interacting pairs Arp 295, M51+NGC 5195, NGC 4676 and NGC 4038/9.



The later stages of a "flat" parabolic passage of "companion" four times as massive as the "victim" galaxy. The companion carries off many particles from the victim, which grows a tail on the opposite side.

contents than igneous glasses for similar SiO_2 contents, and the bottle green microtektites lie on a trend which continues smoothly into the normal microtektites and tektites. The trend for igneous glasses is conspicuously different. Glass thus concludes that the bottle green microtektites are properly named.

But does this conclusion have any bearing on the origin of tektites and microtektites, a matter of long dispute? Glass believes it does. Whatever the precise origin of tektites, most workers seem to agree that they are produced in some sort of impact process—and if this is the case, tektite compositions should give some clue to the nature of the originating material. The most abundant bottle green microtektites have SiO_2 contents of 48 to 54 per cent and on average comprise 61 per cent pyroxene and 22 per cent plagioclase. They also contain normative corundum

and quartz and small quantities of orthoclase and ilmenite. These data suggest that if the originating material were igneous it would have been gabbroic. It would seem, however, that the Al_2O_3 and MgO contents of the bottle green microtektites are much higher than in gabbroic rocks. Furthermore, the bottle green microtektite compositions are not similar to that of any important meteorite group nor to any produced by the mixing of normal meteoritic material with normal tektite material. Nor (pace certain theorists who would place the origin of tektites on the Moon) do they correspond to any important group of lunar material returned to Earth. The obvious conclusion is that if the originating material is on the Earth it must be extremely rare—so rare, in fact, that none has yet been found. The constraint in the origin of tektites and microtektites is thus severe.

Copernicus Published as He Perished

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Nicholas Copernicus was born on February 19, 1473, yet his great work *Revolutions* was only published in March 1543, two months before he died.

THE scientific community all over the world this year will recall that our present conception of the Universe owes its origin to Nicholas Copernicus, who was born exactly half a millennium ago. Throughout the preceding millennium and a half the Earth had been contrasted with the heavenly bodies: they moved and it did not. This motionless Earth was stationed at the centre of a finite sphere, which rotated once a day, carrying with it the bright stars embedded in its otherwise invisible surface. Below this outermost limit of the Universe with its "flaming ramparts", the planets circled at various distances from the centrally located immobile Earth.

This hallowed picture of the cosmos was drastically rearranged by Copernicus. He was the first thinker to set forth cogent reasons for lifting the Earth up out of the centre of everything and putting it where it really belongs: a planet among its fellow planets.

In so doing, he defied common sense. For Earth the planet must move, unlike central Earth. Indeed, Copernicus conferred three motions on the Earth: its daily rotation around its own axis produced the unending succession of day and night; its annual revolution around the Sun caused the cyclical sequence of the four seasons; and a third movement explained the precession of the equinoxes. Yet if the Earth rushed and whirled through space at such awesome speed, it could be objected by common sense, why do its inhabitants not feel its powerful propulsion and ceaseless gyration?

Confronted by an unfamiliar idea, common sense might laugh derisively. But the reaction of entrenched ideology and established religion could be more sinister. A letter written by Copernicus to a clergyman expressed his fear of opposition from the peripatetics and theologians.

Recently we were told that Copernicus was timid, hardly the word to characterize a prudent man living in a harsh age wracked by political warfare and religious strife. When a play produced in a theatre not far from Copernicus's home ridiculed his idea of a moving Earth, he may have recalled with a shudder that Socrates was mocked on the Athenian stage before the popular jury sentenced him to a lethal dose of hemlock.

Some two decades earlier, in the first flush of creative joy at his discovery of the geokinetic astronomy, Copernicus had circulated among selected specialists a few handwritten copies of the brief preliminary draft of his new cosmology. Even then he discreetly withheld his name as author from his so-called *Commentariolus*.

Evidently Copernicus ardently desired to submit his ideas to the scrutiny of contemporaries. Yet he had no wish to become a martyr. Anonymity was one way out of his dilemma. But there was also another.

On November 1, 1536, a highly placed prelate of the Roman Catholic church wrote to Copernicus, urging him to communicate his still unpublished findings to scholars, and

offering to have all of the astronomer's writings copied in his quarters at the cardinal's expense and sent to Rome. A stony silence was Copernicus's response.

By contrast, during the previous year Copernicus had received a visit from an old friend, who was now the secretary of the king of Poland. In this capacity he was in touch with important personages in Vienna, then the capital of the Holy Roman Empire and a pulsating hub of the publishing trade. There was no printing press in that "most remote corner of the Earth", as Copernicus himself described the place where he spent most of his time during the last three decades of his life. Hence the Vienna connexion offered him a golden opportunity.

One method of testing the soundness of an astronomical system is to compare its detailed predictions of what will be seen in the heavens with the observed phenomena as they occur. The logical outcome of a prolonged and extensive investigation, such as Copernicus conducted for nearly half a century, is the computation of a fresh set of astronomical tables. The forecasts derived by extrapolation from these new columns of figures could be critically juxtaposed, not only alongside future observations but also past tables, now submitted to competitive examination.

This was a test which Copernicus welcomed. "For the common advantage of everybody" he was then engaged in preparing an almanac which he was confident was far more accurate than the one in common use. But his busy visitor had to leave before Copernicus could finish his almanac. A copyist was hastily called in, who under these circumstances naturally made some mistakes. These had to be corrected and the gaps had to be filled by a qualified person in Vienna. The royal secretary's accompanying letter stated that Copernicus "for many years has believed that some motion must be granted to the Earth, and asserts that the motion of the

*Rex me in Christo pater et dominus datus clarissimus. accepit hunc et deo humanissimas
et admodum famulantes, quibus in ista vita designata est minister ad testandum libere
meum opusculum elegans fore et ad rem non minus meritis, sed et deo benevolentia
surgens, quae studiosos persequi solet. Ipse quidem et deo et ista opera meo in istis
propter, si modo dignum sit quod, quod a re deo persequi tamquam meritis, quod hunc
liberum me de hunc offi. alij, quibus alij dicit. Ego pro singulis benevolentia
et affertur erga me primum quod me persequi non offit et deo quatenus in me est
promerit, erga in amorem quod persequi non debet persequi et alij erga
Ex. flamberg 27. 15. 91*

€ R 2 0

obsequium

Nicolaus Copernicus

Fig. 1 Facsimile of a letter written and signed by Copernicus.

Earth is not felt". Perhaps this shock to common sense, perhaps the lacunae and errors, perhaps the death of the king's secretary, prevented the publication of Copernicus's almanac. It has disappeared without leaving a trace.

In deciding what should and what should not be made public, Copernicus followed the lead of the ancient Pythagoreans. Because he admired their code of conduct, his astronomical system was often identified with theirs, although the resemblances are slight and the differences significant. As a closely knit brotherhood, the Pythagoreans believed in "entrusting the secrets of philosophy only to faithful friends and associates, and not committing those secrets to books nor disclosing them to everybody". While making his almanac available for publication, Copernicus did not turn over the novel principles on which his new astronomical tables and almanac were based. By withholding his reasoning, which he realized was in conflict with traditional ideas and common sense, Copernicus felt that he would "provoke no dispute among philosophers".

His best friend disagreed, for he knew that current astronomical theory badly needed overhauling—and the ecclesiastical calendar was woefully out of joint. Aware of Copernicus's exceptional talent and unremitting labours, he kept on pressing for complete publication of the new system.

There were two works in the field which could serve as possible precedents. The Alfonsine Tables, prepared at the expense of and named in honour of a thirteenth century king of Castile, formed the model preferred by Copernicus. This mediaeval Spanish product reported no observations on which the Tables were based, and enunciated no rules or principles by the light of which they had been drawn up. In spite of their skeletal shape, they were entirely satisfactory to the practical astronomer (and astrologer), who had no particular interest in the underlying theory. By contrast there was Ptolemy, the greatest of the ancient Greek theoretical and practical astronomers. His *Syntaxis* (often miscalled the "Almagest") was crammed full of observations, theorems, proofs, deductions, and diagrams. His illustrious example was therefore to be shunned, in Copernicus's judgment. For how could acrimonious controversy be avoided if geokineticism were to be fully exposed to public view?

But Copernicus's friend could not be persuaded or silenced. He insisted on the difference between science and politics. In delicate negotiations undertaken to conclude a vexatious war or regulate troublesome public affairs, secrecy was justified as a temporary expedient. But once secluded discussions were successfully terminated, the dark curtain would be raised to disclose the new situation. On the other hand, if Copernicus behaved like a special plenipotentiary, hiding his principles and displaying only their results as a set of stark naked tables, who thereafter would ever be able to unravel the intricate details and lay bare the underlying conceptions? Would not the scientific community be forever deprived of the sorely needed light which Copernicus for purely prudential reasons was hiding under a bushel?

This seesaw dialogue might have batted the ball back and forth indecisively and interminably, had not a fresh player entered the stalemated game from the outside world. A young German professor of mathematics arrived on the scene, attracted by rumours of revolutionary innovations and eager to assess them at first hand for himself. Received with open arms and granted access to the concealed manuscript, within a brief span of time he became thoroughly convinced that the gossip he had heard in far-off Germany was no exaggeration. Here in Copernicus's closely reasoned and thoroughly documented presentation lay, like a restive foetus, the long-awaited reformation of astronomy.

Yet no amount of persuasion, however earnest or skilful, could induce Copernicus to release his own manuscript to the press. He raised no objection, on the other hand, to his disciple's swift composition of a *First Report* about the Copernican astronomy. This was rushed to a printer in the

Hanseatic town of Gdańsk, where it was published in March 1540. Copies were dispatched at once to influential friends everywhere. Its author, recognizing that it "could be considered heretical (as the monks would say)", waited anxiously for the explosion that he anticipated. None occurred. Instead, a second edition of his *First Report* was issued at Basel in 1541. Still no detonation was heard.

At last Copernicus was convinced. He finally consented to the publication of his manuscript, preceded by a magnificent plea for freedom of thought and expression. In particular he besought the bibliolators to refrain from twisting Scripture to the detriment of science. Lactantius was no mean writer, but he made fun of those pagans who maintained that the Earth was round. The implication was clear. The theologian had his own important task, but unless he also had additional training that made him an expert in the mathematical sciences too, he was not qualified to sit in judgment on the new astronomy. That was written for specialists. This is the meaning of Copernicus's most famous utterance ("*Mathematica mathematicis scribuntur*"), which carries no overtones of condescension or contempt for the untutored, as has been mistakenly charged by his would-be detractors. After all, Copernicus wrote his *Revolutions* in Latin (as a century and a half later Newton, that greatest of Copernicans, did with his even more momentous *Principia*), and for the untutored anything in Latin was, of course, a closed book. When Copernicus decided to publish, he addressed himself to his compeers.

He laid no claim to originality. Far from professing to be the first thinker to have recognized the true cosmic status of the Earth as a planet in motion, he delved into whatever ancient writings happened to be available in a quest for his own precursors. From them he obtained, not his ideas, but a protective shield for his ideas. The point is that these were not really new. They were quite respectably old, but somehow they had become buried beneath the accumulated debris of the centuries, and merely had to be disinterred and cleaned up.

Not as a revolutionary, nor as a subversive, but as a resuscitator of forgotten wisdom, did Copernicus tardily appear in public. His youthful German collaborator made a fair copy of Copernicus's precious autograph (now splendidly reproduced in photofacsimile), and elicited from the Duke of Prussia requests addressed on September 1, 1541, to the Elector of Saxony and the University of Wittenberg for permission to publish Copernicus's *Revolutions*.

If it is true that science owes the publication of Newton's *Principia* to Edmond Halley, it is equally true, as George Joachim Rheticus later declared, "Had I not visited Copernicus, his work would absolutely not have been published".

It was Rheticus who made the arrangements with an active and prosperous shop in Nuremberg to print Copernicus's masterpiece. But Rheticus could not finish reading the proofs and doing the editing because he had to leave for the University of Leipzig, which had just appointed him professor of mathematics.

His place was taken by a local minister, whose hobby was the mathematical disciplines. His philosophy of science was diametrically opposed to Copernicus's, as he well knew from the letters they had already exchanged. Taking advantage of Copernicus's grave illness in remote Frombork and of his own inside position as editor, he surreptitiously introduced into the front matter of the *Revolutions* an unsigned foreword wholly foreign to Copernicus's intention. Whereas the astronomer firmly believed that he had disclosed an essential feature of the physical universe, his editor presented it as though it were merely a convenient device for making computations. It was not necessarily true, and might even be false.

Thus speciously accoutred, Copernicus's *Revolutions* came off the Nuremberg press in mid-March 1543. By the time a copy reached the author in distant Frombork, he was lying barely conscious on his sickbed. Copernicus never knew about the intrusive, well-intentioned, but misleading foreword, for, on May 24, 1543, he perished as he published.

Real Space Crystallography

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We have discovered an image plane technique of crystallographic analysis which achieves spatial resolution of structural defects and diffraction information, reveals a lack of a centre of symmetry, requires only minute crystals and is rapidly executed.

IN spite of its achievements X-ray crystallography has a number of limitations. One is the loss of phase information in X-ray diffraction so that heavy atom derivatives of the crystal under investigation have to be obtained¹; another is the limitation on crystal size which arises from the relatively weak interaction of X-rays with matter. If the crystals are too small, too imperfect or combined in a mixture of too many different components, X-ray methods break down; applications where problems have arisen are in the crystallography of biological molecules; martensitically transformed materials; polytypes of layer structures; plagioclase minerals, and the great variety of non-stoichiometric oxides. Electron diffraction which can in principle overcome these difficulties has until now generally involved too many mathematical problems to be useful², but recent developments in both experimental technique and interpretation indicate that electron microscope observations can easily yield the point groups of difficult crystal structures and make complete structure determinations possible.

Electron and X-ray diffraction are very different. Apart from the obvious differences in strength of interaction with a crystal and the resolution of the two techniques two other properties are important for the new approach. At 100 keV the electron wavelength is ~ 0.04 Å so that the Bragg angles are very small for electron diffraction. Moreover, owing to the existence of electron lenses observations can be made either in the image plane (for which there is strictly no X-ray equivalent although Lang topography is similar) or in the back focal plane of the objective lens (similar to the conventional X-ray diffraction pattern). If an aperture is introduced in the back focal plane to encircle either the direct or a diffracted path diffraction information may be recorded in the image plane. Because transmission specimens in the electron microscope

have to be thin (~ 2000 Å) they bend very easily through angles large compared with the Bragg angle. The diffraction contrast in the specimen image is therefore in the form of lines joining parts of the crystal which diffract similarly (Fig. 1). To take a simple case, consider plane electron wave-fronts incident on a cylindrically bent crystal with a radius of curvature R . If there is appreciable diffraction when the waves are incident

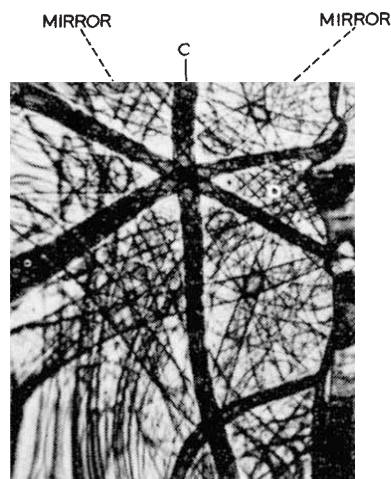


Fig. 1 100 keV bright field bend contour pole of 3-fold symmetry in Ta_2O_5 (nominal composition). The pattern terminates along the irregular grain boundary at the right hand side of the figure.

at the Bragg angle (θ_B) a dark line appears at that part of the crystal in a bright field (aperture encircling the direct beam) picture. At a distance $2R\theta_B$ away another line is formed by Bragg reflexion in the opposite sense. In some cases the region between the pair of contours is dark and this is due to the excitation of strongly absorbed lattice waves concentrated on the atomic planes³ (contour C Fig. 1).

From this simple explanation it is clear that the specimen area shown in Fig. 1 is in the shape of a slightly distorted

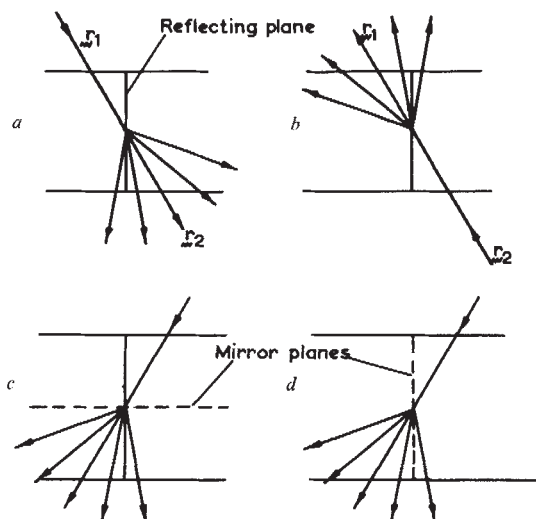


Fig. 2 Use of the reciprocity theorem together with crystal symmetry to deduce bend contour symmetries.

saucer over much of the field of view. As a result of the specimen geometry it is possible to tilt the specimen so that the electron illumination falls on it along any required direction within the range of the goniometer available. Commercially available electron microscopes may be equipped with goniometer stages permitting tilts of $\pm 60^\circ$ about any axis in the foil plane with specimen shift of only 1–2 μm .

Point Group Determination

The centres of various bend contour pairs in Fig. 1 are crystallographic zones. In practice therefore a prominent

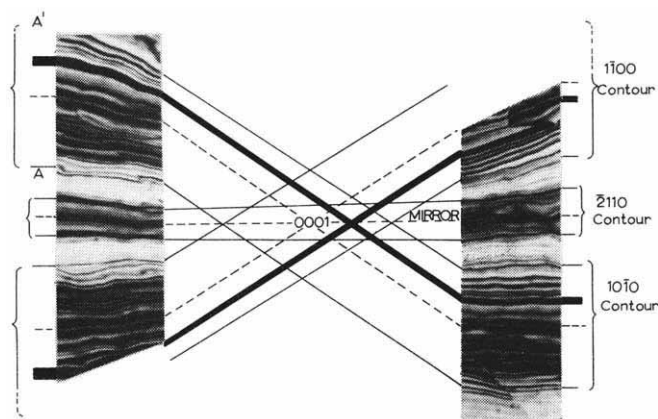


Fig. 3 Bright field bend contours at 100 keV on a polytype of molybdenite showing violation of Friedel's Law.

contour in the field of view is chosen and the specimen rotated and tilted until this zone intersects another prominent zone. The symmetry properties of this intersection, which is known as a zone axis pattern, are then examined. The pole in Fig. 1 may be seen to arise from an axis of three-fold symmetry with

mirror planes every 60° and therefore the crystal is either trigonal or cubic. Subsequent rotation and tilt experiments reveal that the specimen has the point group $3m$. Owing to high intensity of the electron beam and the ease with which the goniometry may be performed it is possible to arrive at the conclusion in the course of an experiment lasting about 30 minutes. Clearly n fold axes and mirror plates can be detected easily. What is more surprising is that it is also possible to tell from bright field micrographs whether the crystal has a centre of symmetry.

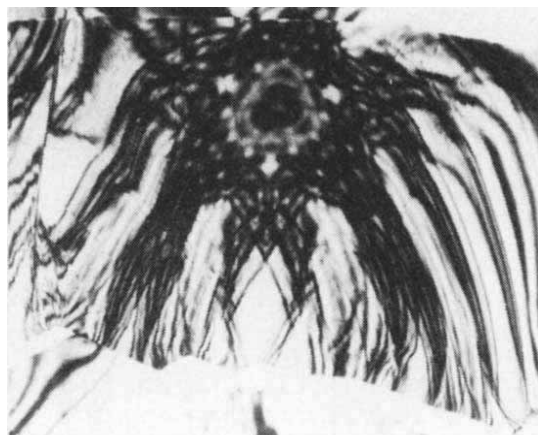


Fig. 4 Image detail in 3-fold and 6-fold zone axis patterns of different polytypes of MoS_2 .

There has been misunderstanding over this point⁴ and so we shall re-examine the argument which is based on the so-called reciprocity theorem. According to this theorem⁵, if a signal is detected at a point r_2 when a source is placed at another point r_1 , then the same signal in amplitude and phase would be detected at r_1 if the source were placed at r_2 . In bright field the situations shown in Fig. 2 *a* and *b* are thus equivalent as are also *c* and *d* on addition of the mirror planes shown. By a minor adjustment of the argument we imagine the angle of incidence fixed and the foil bent. To a good approximation, therefore, we expect symmetric contours in bright field when *c* and *d* apply.

In the case of basal foils of a non-centred polytype (2T) of molybdenite, the mirror in Fig. 2 *c* is absent. $\{\bar{2}110\}$ planes have the mirror in Fig. 2 *d* and yield symmetric contours (Fig. 3), but $\{1\bar{1}00\}$, lacking the extra mirror, give asymmetric contours (a violation of Friedel's Law). After tilting through the (0001) pole the asymmetry of the $\{\bar{1}100\}$ contours is reversed with respect to the $\{\bar{2}110\}$ contour, verifying the significance of this observation. Note that we have not made use of the projection or zero layer approximation which is often made in the theory of electron diffraction. Had we made this approximation the mirror plane of Fig. 2 *c* is added and hence symmetric contours would result in bright field. In molybdenite the large *c* spacing makes the effect of other layers of the reciprocal lattice particularly important.

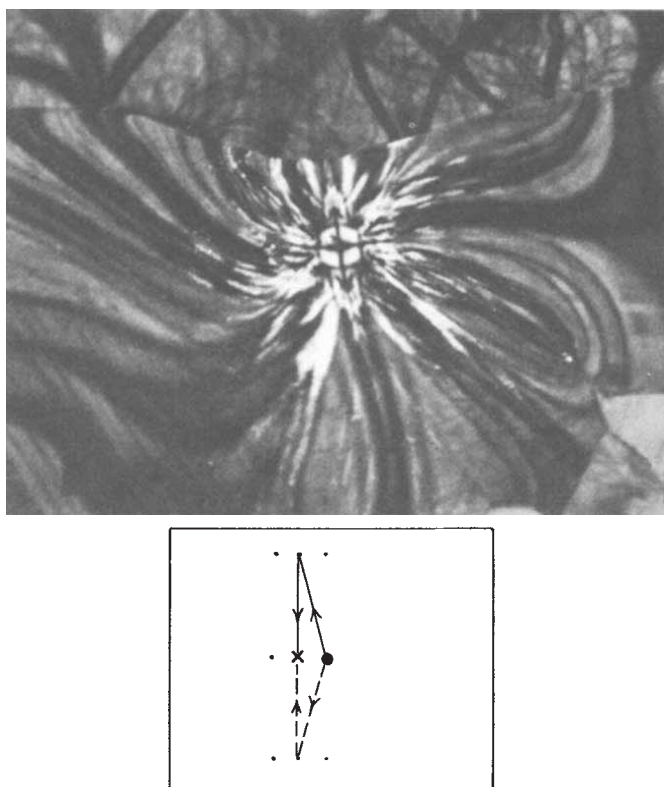


Fig. 5 Dark field electron micrograph obtained by encircling a forbidden spot occurring in a $(10\bar{1}0)$ pattern. The sketch shows a proposed pair of double diffraction paths.

Zone axis patterns shown in Fig. 4 easily discriminate between polytypes with three-fold and six-fold axes, a difficult problem by conventional techniques⁶.

Because the area of interest is continuously in view while the specimen manipulation is performed, the diffraction information can always be obtained from this area, a considerable advantage over selected area diffraction techniques. Moreover, in view of the dynamic nature of the experiments two important benefits are obtained. First, the operator can test hypotheses about symmetry during the course of the experiment, by large tilts as well as by study of zone axis patterns. Second, imperfections in the diffracting area are much easier to detect as a series of diffracting conditions is carried past in quick succession than by a few static results which may in any event have been obtained under unfavourable conditions. There are two techniques in electron microscopy which are

essentially similar to the one described here but differ in requiring flat specimens. These are the study of Kikuchi line patterns resulting from inelastic and thermally scattered electrons, and the convergent beam technique. The first is dependent on the presence of Kikuchi lines which are not at all common in imperfect crystals. Moreover, Kikuchi lines are invariably less detailed than bend contours and much harder to interpret. Convergent beam techniques as practised at present lack the resolution possible here and require special electron diffraction apparatus.

Space Group Determination

To take the next step in structure determination it is necessary to identify the forbidden reflexions. Owing to multiple scattering of electrons, which occurs even in very thin foils, diffraction spots can appear in forbidden positions. An experimental technique was devised for identifying this phenomenon during our work on Ta_2O_5 . On rotating a foil about the *c* axis from $(11\bar{2}0)$ to $(10\bar{1}0)$ we found the spacing of spots in the *c* direction halved. Suspecting double diffraction, we used a very small objective aperture to encircle one of the two addi-

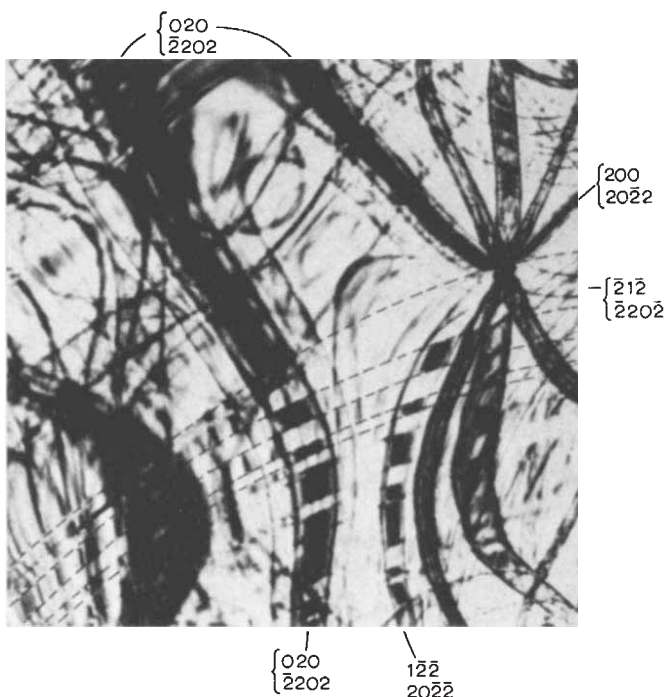


Fig. 6 Bright field bend contours at 100 keV in a multiply twinned Ta_2O_5 specimen. Changes in the form of the 020 contours are due to their transformation to $2\bar{1}2$ in the twinned crystals.

tional spots nearest the centre of the pattern. The resulting dark field micrograph is shown in Fig. 5. Strong diffraction is limited to the region of $[10\bar{1}0]$ zone axis, confirming the hypothesis. A possible diffraction path is indicated in the figure. Further information is visible in this result. The black cross at the centre of the pattern implies that the electron beam is incident either perpendicular to a screw axis of the crystal structure or parallel to a glide plane. Under these circumstances the possible routes to the forbidden positions may be arranged

in pairs contributing equal diffracted amplitudes of opposite sign⁷. The space group of the new form of Ta_2O_5 is found to be $R\bar{3}2/c$.

Minimum Crystal Size

The limitations on the nature of specimens which are suitable for the new method of analysis are not too severe. They concern the curvature and microstructure of the foils. If a foil only bends through a fraction of a Bragg angle in the field of view it would be too laborious a matter to piece together a

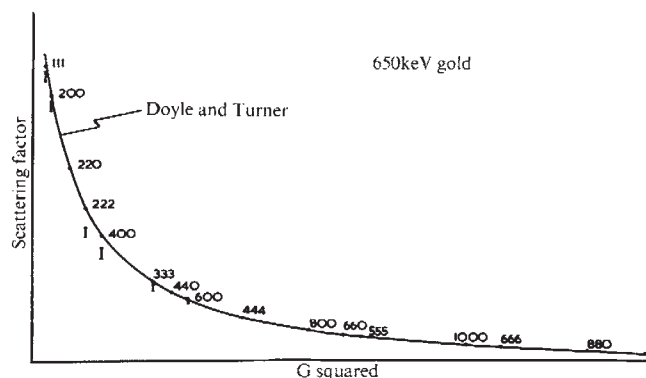


Fig. 7 Atomic scattering factor of gold deduced from bright field and dark field contours of 111 and 200 reflexions. The experimental results are indicated by error bars.

contour map. In fact a misorientation of about 5° is a convenient working minimum. Flatter foils are generally suitable for Kikuchi line techniques. To make clear observations of symmetry it is necessary to work in relatively perfect regions and this imposes a limitation on the grain size of a polycrystalline material or the cell structure or dislocation density of a deformed material. It has been ascertained by experiment that a prominent contour is recognizable down to widths $\sim 2000 \text{ \AA}$ so that a reasonable field of view in a suitable curved specimen can be as low as $0.5 \mu\text{m}$ for an equi-axial microstructure. This observation is compatible with the lateral coherence of the electron beam, about $100 \text{ \AA}$. The limiting situation may be expected when a foil changes its orientation by 0.1 Bragg angle in this distance. Much better resolution is, however, possible in particular cases; an example is shown in Fig. 6. The trigonal phase ($\bar{3}m$) of Ta_2O_5 shown has been found by experiments in larger areas to contain twin boundaries. Many of the bend contours are unaffected by the twinning and run continuously from one phase to the next. An exception is the (002) contour that is notable for its dark centre. On passing into a twinned region the dark centre vanishes but the second line, and some of the other higher order lines, are continuous. Thus twins may be detected in regions less than $500 \text{ \AA}$ wide.

Atomic Arrangements

Our ultimate objective is to establish atomic arrangements in crystals of unknown structure. That there is a lot of information in micrographs such as Fig. 4 is obvious. Where the diffraction occurs from relatively close lattice planes the contours take the form of simple pairs of lines. In such cases, the theory of electron diffraction is not very different from that of X-ray diffraction and structure factors may be approximately determined from the visibility of the lines. This proposition has been tested in the case of mica, in the bend contour pattern.

All strong lines are those which have large structure factors; none of the visible lines has a small structure factor. No doubt some reflexions with large structure factors do not give strong lines because of the combined effect of weak reflexions that are simultaneously excited. One effect of this type has been termed the critical voltage effect⁸ and has been used to yield valuable information about the crystal potential⁹.

More interesting are the contours corresponding to high voltages, high atomic numbers and large lattice periodicities, and to high symmetry zone axes. They are quite complicated in form and contain more detailed diffraction information. The first example of this sort to be thoroughly studied was the case of the (nmn) reflexions from gold at approximately 600 keV . The method of extracting information from the bend contours was based on the dispersion surface construction^{10,11}. These are constant energy surfaces in reciprocal space equivalent to the Fermi surface for conduction electrons. Each point on a given surface corresponds to a particular direction of incidence, each surface to a Bloch wave in the crystal. The reciprocal of the separation of simultaneously excited points

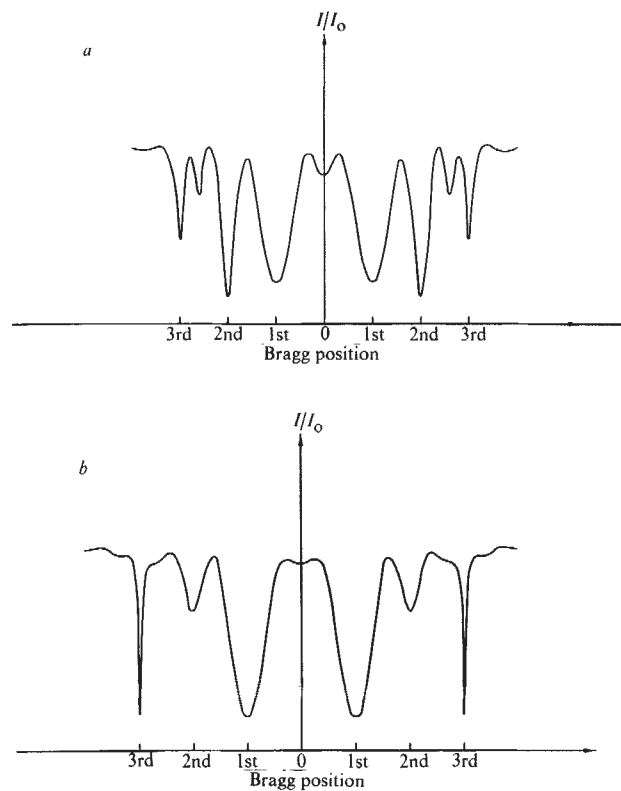


Fig. 8 Calculated contours for the $(10\bar{1}0)$ reflexion of (a) 2H_1 and (b) 2H_2 MoS_2 for a specimen thickness of $500 \text{ \AA}$ and an accelerating voltage of 100 keV .

on the dispersion surface gives the depth periodicity in the crystal associated with energy transfer from one Bloch state to another. Thus it is possible to read out information from the bend contours about the dispersion surfaces and hence deduce the crystal potential. Results from the atomic scattering factor of gold are compared with the best available computed data in Fig. 7 (C. J. Richards, unpublished work).

More recently we have performed calculations for two hexagonal polytypes of molybdenite with the same space group but different atomic arrangement. The form of the $\{11\bar{2}0\}$ contours is essentially unchanged in the two cases and can be used to fix the foil thicknesses. The $\{10\bar{1}0\}$ and $\{\bar{1}103\}$ contours are quite different, however, and can be used to distinguish between the two atomic arrangements. The results of calculations for the $(10\bar{1}0)$ contour are shown in Fig. 8.

Where the diffraction problem is essentially one dimensional the many beam theory of Howie and Whelan has been used in calculations. However, the previously intractable problem of zone axis orientations has recently been solved by semiclassical methods^{13,14}, and the method of solution is not only quite straightforward but the process may be inverted to yield directly the atomic potential from experimental observations. We are now attempting a complete structure determination by the use of these recently evolved techniques.

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Tetrahydroisoquinoline Alkaloids: *in vivo* Metabolites of L-Dopa in Man

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Tetrahydroisoquinoline alkaloids have been unequivocally demonstrated *in vivo* for the first time in parkinsonian patients on L-dopa treatment. Their biosynthesis may have a profound bearing on how L-dopa acts.

ALTHOUGH the therapeutic effect of L-dopa in parkinsonism is no longer in doubt, its mode of action remains ill-understood. Early explanations in terms of simple replacement of deficient

dopamine no longer seem wholly tenable. To account for all the observed clinical and biochemical phenomena, more sophisticated hypotheses have been postulated involving other pharmacologically active compounds derived from minor pathways of L-dopa metabolism (for review, see ref. 1). One such group of compounds, the tetrahydroisoquinolines, formed by the Pictet-Spengler condensation² of dopamine with an aldehyde, have been proposed by Sourkes³ on theoretical grounds. Although many Schiff base derivatives of this type develop readily *in vitro* (for review, see ref. 4), evidence for their *in vivo* production in animal tissues is, at best, equivocal⁵. We report here the identification, in significant concentration, of two of the alkaloids, salsolinol and tetrahydropapaveroline, in the urine of patients with Parkinson's disease during oral L-dopa treatment.

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Analysis of Alkaloids

Much evidence (for review, see ref. 4) suggests that the formation of these compounds is facilitated by ethanol: salsolinol results from the reaction between dopamine and the ethanol metabolite, acetaldehyde; ethanol and acetaldehyde both appear to promote the synthesis of tetrahydropapaveroline which readily condenses *in vitro* from dopamine and the aldehyde deriving from the oxidative deamination of a second molecule of dopamine (Fig. 1).

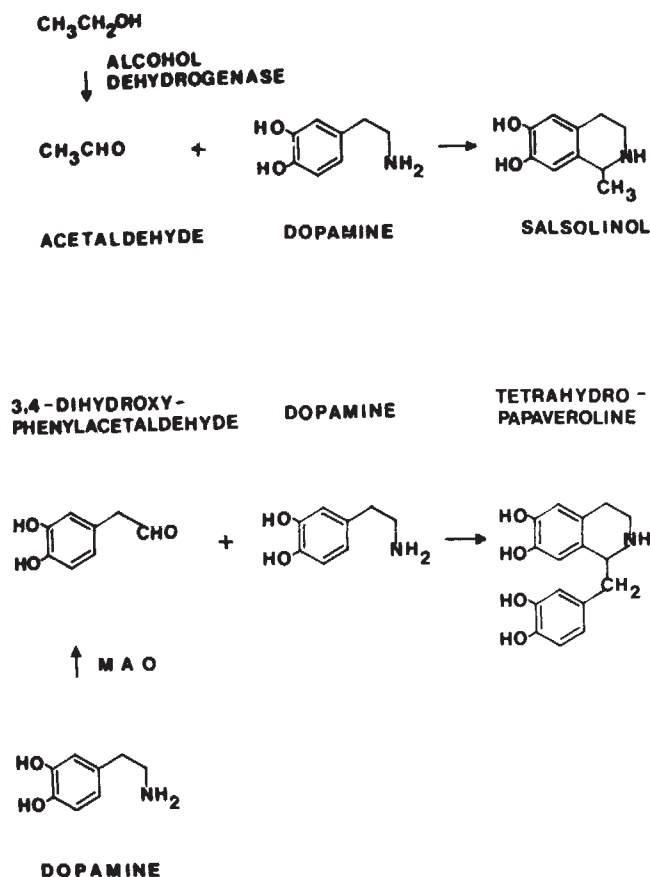


Fig. 1 Formation of salsolinol and tetrahydropapaveroline.

We used gas chromatographic methods to measure the concentration of these compounds in urine (ref. 6 and K. P. Wong, C. R. J. Ruthven and M. S., submitted for publication) from parkinsonian patients on L-dopa treatment before and immediately after ethanol ingestion. Four male patients (average age 56 years) who had responded well to an optimal oral daily dose of L-dopa (3–4 g) and understood the nature of the experiment, ingested either 75 ml. absolute alcohol (test), or 75 ml. water (control), made up to 160 ml. with water and flavouring at 0930 h over a 30 min period on two separate days. Urine samples were collected for the periods 0–3, 3–6, 6–9 and 9–12 h after the drink, acidified to pH 1–2 with hydrochloric acid and stored at -15°C until analysis.

Appreciable concentrations of salsolinol and tetrahydropapaveroline detected gas chromatographically as their pentafluoropropionyl (PFP) derivatives (Fig. 2) were excreted throughout the period under study (Fig. 3). We cannot, for a number of reasons, obtain any precise rate of synthesis from the serial 3 h excretion values from which the total 12 h output was calculated. These measurements, which varied widely,

reflect the individual response of particular patients in whom dosage of L-dopa and its time of administration, gastric

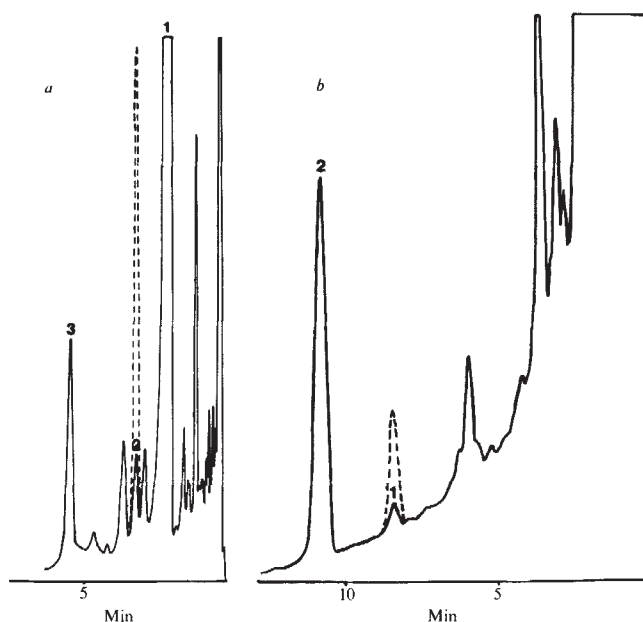


Fig. 2 *a*, Gas chromatogram of urinary extract containing salsolinol. 1=dopamine; 2=salsolinol (urinary output of salsolinol, —); same urine sample after addition of internal standard, - - -; 3= γ -lindane. The concentration of salsolinol in this particular urine sample was approximately 300 ng/ml. and the amount injected approximately 60 μg . *b*, Chromatogram of urinary extract containing tetrahydropapaveroline. 1=tetrahydropapaveroline (urinary output of tetrahydropapaveroline, —); the same urine sample after the addition of internal standard, - - -). The concentration of tetrahydropapaveroline in this particular urine sample was approximately 150 ng ml. $^{-1}$ and the amount injected approximately 750 μg . 2=dieldrin. Columns of acid-washed alumina^{31–33} (0.5 g) were prepared from a slurry in 5 ml. sodium acetate reagent (0.2 M sodium acetate, 0.2% (w/v) EDTA, 0.1% (w/v) ascorbic acid, adjusted to pH 8.4 with 0.1 N sodium hydroxide). Duplicates (10 ml.) of each urine specimen were analysed by a method to be published in full elsewhere (K. P. W., C. R. J. R., and M. S.). To one of the duplicates had been added salsolinol (10 μg) (salsolinol hydrobromide purchased from Ralph N. Emanuel, Ltd., Wembley) and tetrahydropapaveroline (5 μg) as internal standards. Ascorbic acid (0.2 ml., 5% (w/v) freshly prepared) and EDTA (0.1 ml., 10% (w/v)) were added, in addition, to all duplicates³⁴, which were then adjusted to pH 8.4 and passed through the alumina column. After the column had been washed with sodium acetate reagent (5 ml.) and water (10 ml.), salsolinol and tetrahydropapaveroline were eluted with 0.5 M acetic acid (2.5 ml.) then water (2.5 ml.). Subsequent fractions, eluted with 0.5 M acetic acid, contained neither compound. A portion of the combined eluate (1 ml.) was taken to dryness and reconstituted in 0.2 ml. methanol/0.01 N HCl (1 : 1). One twentieth of this was used for analysis of salsolinol and one half for analysis of tetrahydropapaveroline. Pentafluoropropionyl (PFP) derivatives were prepared for gas liquid chromatography using pentafluoropropionic anhydride (ref. 6 and K. P. W., C. R. J. R., and M. S.). Immediately before injecting the samples, excess reagent was blown off under nitrogen and the derivatives reconstituted in dry ethyl acetate (0.1 ml.) containing reference standards of 1 μg ml. $^{-1}$ γ -lindane for salsolinol estimations and 2 μg ml. $^{-1}$ dieldrin for tetrahydropapaveroline. Recovery studies were carried out on PFP derivatives of salsolinol (100 ng) and tetrahydropapaveroline (500 ng). Samples were run on a 'Perkin-Elmer model 900' gas chromatograph with the following column conditions: carrier gas, nitrogen, flow rate 40 ml. min. $^{-1}$; injection port temperature, 260 $^\circ\text{C}$; manifold temperature, 260 $^\circ\text{C}$; electron capture detector temperature, 300 $^\circ\text{C}$. A 12 foot glass column (i.d. 2 mm) packed with 10% SE54 on 'Gas Chrom Q', 80–100 mesh, was used at 210 $^\circ\text{C}$ for salsolinol and 230 $^\circ\text{C}$ for tetrahydropapaveroline estimations. Quantification was achieved by measuring peak heights and correcting for errors in injection using the reference standards. Salsolinol and tetrahydropapaveroline concentrations were calculated by comparison with the duplicate sample containing internal standards.

emptying and gastrointestinal absorption all differed. Also both compounds are labile in solution, so that standards must be freshly prepared in 0.01 N hydrochloric acid, and their PFP derivatives are unstable. They can be stored deep-frozen overnight in the presence of the pentafluoropropionic anhydride but, once dissolved in ethyl acetate for gas chromatography, are easily hydrolysed. Despite their lability in standard solution, however, they tend to be considerably more stable in urine. Fresh 3 h urine samples were therefore obtained from another four parkinsonian patients, two male and two female, on L-dopa ($3\text{--}5\text{ g day}^{-1}$), and analysed immediately. Although salsolinol excretion was somewhat higher, the data were not too dissimilar from those obtained in the previous experiment, indicating that deterioration during storage was at an acceptable level. Tetrahydropapaveroline was completely absent from a proportion of the stored specimens for reasons not yet determined. Another quantification difficulty was the wide recovery range (20–100%) of these compounds from different urine samples, for unknown reasons, and the values therefore must be considered only as semiquantitative estimates. We hope to obtain more exact information by the use of a reverse stable isotope dilution technique combined with gas chromatography–mass spectrometry⁷.

Our data demonstrate the *in vivo* production of both salsolinol and tetrahydropapaveroline in man for the first time, and therefore, in order to establish their identity as precisely as possible, mass spectrometric studies were carried out. Standard spectra of salsolinol and tetrahydropapaveroline, as their fully substituted PFP and trimethylsilyl (TMS) derivatives, were recorded on both 'AEI MS12' and 'Varian-MAT 311' mass spectrometers by gas chromatographic inlet. The alkaloids were detected in urine extracts using the technique commonly known as 'mass fragmentography'⁸ (Fig. 4), the rapid accelerating voltage switching between values required to focus fragment ions characteristic of a particular compound during the course of gas chromatographic elution. This was effected for the tri-PFP derivative of salsolinol using m/e 617 (M^+) and 602 ($M-15$) ('Varian-MAT 311' by 3% OV-1) and m/e 617 and 603 (MS-12 by 3% OV-17) and as the tri-TMS derivative using m/e 380 ($M-15$) and 381 (MS-12 by 3% OV-1). The penta-PFP derivative of tetrahydropapaveroline was detected by single ion monitoring of the base peak, m/e 602 ('Varian-MAT 311' by 3% OV-1) and m/e 603 (MS-12 by 3% OV-17) and as the penta-TMS derivative monitoring m/e 380 and 381 (MS-12 by 3% OV-1). These data appear to provide substantial evidence for the presence of the alkaloids in urine.

It is not yet possible to isolate a sufficient amount of each from urine for complete mass spectra to establish their absolute identity. Thus a finite (but for salsolinol, at least, extremely remote) possibility exists that a very close analogue co-elutes and contains similar ion fragments in a similar ratio. The risk of false identification is slightly greater with tetrahydropapaveroline where the fragment ions used for detection arise from only one half of the molecule. We do not, however, entertain any doubts as to their identity.

Because of these technical difficulties such qualitative precision can only be matched quantitatively when deuterated internal standards have been synthesized⁹. Nevertheless, it seems justifiable to draw certain conclusions from our semiquantitative data. There appears to be an increase in salsolinol output in the 12 h period following ethanol ingestion (Fig. 3a). Ethanol may have a similar effect on tetrahydropapaveroline excretion (Fig. 3b), although the pattern is not so consistent and the data too fragmentary to draw valid conclusions.

The relatively small output of tetrahydroisoquinolines detected during the present series of experiments gives little indication of total production and may well represent but a small fraction of the whole. The alkaloids identified may be short-lived intermediates in a system changing rapidly by routes of metabolic degradation which may be numerous and

complex. An attempt to identify possible conjugation pathways by the use of a sulphatase/glucuronidase preparation ('Suc d'*Helix pomatia*') was not successful; the prolonged period of incubation involved resulted in breakdown of the compounds. Hydrolysis was therefore carried out by heating in a boiling water bath (pH 1, 20 min) portions of (a) all four fresh urine samples and (b) all urine samples from patient 4 in the ethanol experiment. Internal standards of salsolinol and tetrahydropapaveroline were added to duplicates before hydrolysis to enable adjustments to be made for losses on heating. Significant increases were not observed, however, in

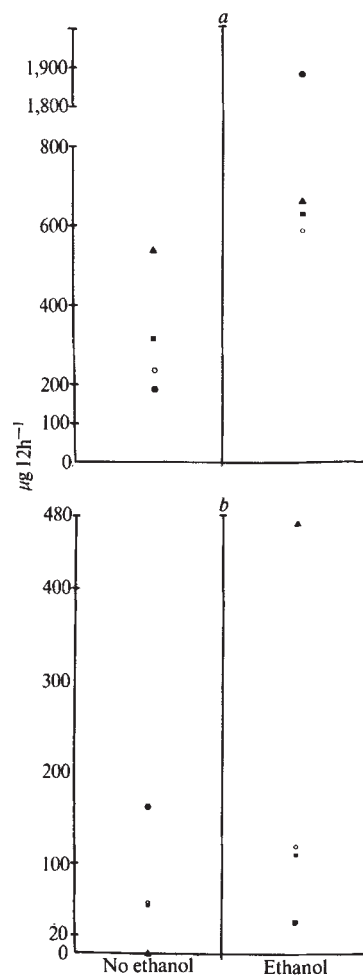


Fig. 3 Urinary excretion of (a) salsolinol and (b) tetrahydropapaveroline ($\mu\text{g } 12\text{ h}^{-1}$) before and after ethanol ingestion (see text). Each symbol represents a different subject.

either set of samples after hydrolysis. Unless the conditions adopted were unsuitable, neither alkaloid appears to be excreted to any large extent as a sulphate or glucuronide conjugate.

The Pictet-Spengler Reaction

The Pictet-Spengler reaction involves the condensation of β -arylethylamines with carbonyl compounds². Only amines with a sufficiently high electron density at the point of ring

closure will cyclize with an aldehyde, as in the present experiment, to form a 1,2,3,4-tetrahydroisoquinoline. This molecular prerequisite is fulfilled with 3-hydroxylated β -phenylethylamines and with β -(3-indolyl)ethylamines. A reaction of this type is used extensively for the fluorescence histochemical localization of biogenic amines in tissues where the carbonyl agent used is formaldehyde gas¹⁰.

Dopamine, of the biogenic amines which have so far been investigated, enters into the most rapid *in vitro* reaction with acetaldehyde^{11,12}. By demonstrating unequivocally the urinary excretion of salsolinol, we have now been able to show that this reaction occurs similarly *in vivo*.

Salsolinol

Although the presence of urinary salsolinol after ethanol ingestion conformed with prediction, we were surprised to detect a relatively high output of alkaloid in these dopa-treated patients even before ethanol administration. This finding suggests an endogenous source of circulating acetaldehyde, the origin of which is speculative at the present time. There is evidence that ethanol is formed endogenously in rat, rabbit and man¹³⁻¹⁵, perhaps in the gastrointestinal tract, and this seems to be the most likely source of this acetaldehyde. Urinary salsolinol output after L-dopa administration thus presumably mirrors endogenous acetaldehyde production and may be useful on this account. Even in the absence of exogenous L-dopa, trace amounts of salsolinol may be excreted. In the urine of two young normal adult subjects, one male and one female, small peaks were present in the appropriate position on the chromatogram corresponding, respectively, to excretion values of 29 and 25 μ g per 24 h; their identity has not yet been confirmed mass spectrometrically.

Relatively little is known of the pharmacology of salsolinol. Both it and its analogue formed from the condensation of dopamine and formaldehyde seem to possess certain properties in common with the catecholamines (see ref. 4). It is entirely possible that the formaldehyde analogue itself is synthesized *in vivo*; there are indications that methanol, the formaldehyde precursor, is generated in the organism¹⁵⁻¹⁷ in a similar manner to ethanol¹³⁻¹⁵ although the function of both is quite unknown.

Tetrahydropapaveroline

Tetrahydropapaveroline was first studied by Laidlaw¹⁸ who identified its hypotensive action in the experimental animal. This action has since been studied by others who have also shown that the compound is a β -adrenergic agonist (see ref. 4).

Hypotension, as an important and occasionally severe side-effect of L-dopa therapy in parkinsonism, has been the subject of considerable interest and speculation. There is evidence to support a central mechanism (see ref. 1), but Calne *et al.*¹⁹ found that hypotension was a less prominent feature of the presumably wholly central clinical response of patients treated with L-dopa plus a peripheral decarboxylase inhibitor. Whether the "peripheral" component derives directly from some action of dopamine itself²⁰, or one of its metabolites, remains to be decided.

The complex actions of dopamine on the human circulatory system have been the subject of a recent review²¹. Its effect is predominantly on the α -adrenergic receptor, but it also acts on specific dopamine receptors in the renal and mesenteric vasculature. In addition, although to a lesser extent, it stimulates β -adrenergic receptors, and in view of our results it seems quite possible that the generation of tetrahydropapaveroline contributes to, or is responsible for, such an effect.

It has been known for many years that there is a peripheral component to parkinsonian tremor; this physical sign is potentiated both by emotional tension and adrenaline administration, an effect unmitigated by the α -adrenergic blocking drug phentolamine, but abolished by β -blockade²². Very occasionally, L-dopa may precipitate an acute, transient episode of parkinsonian tremor with simultaneous relief of

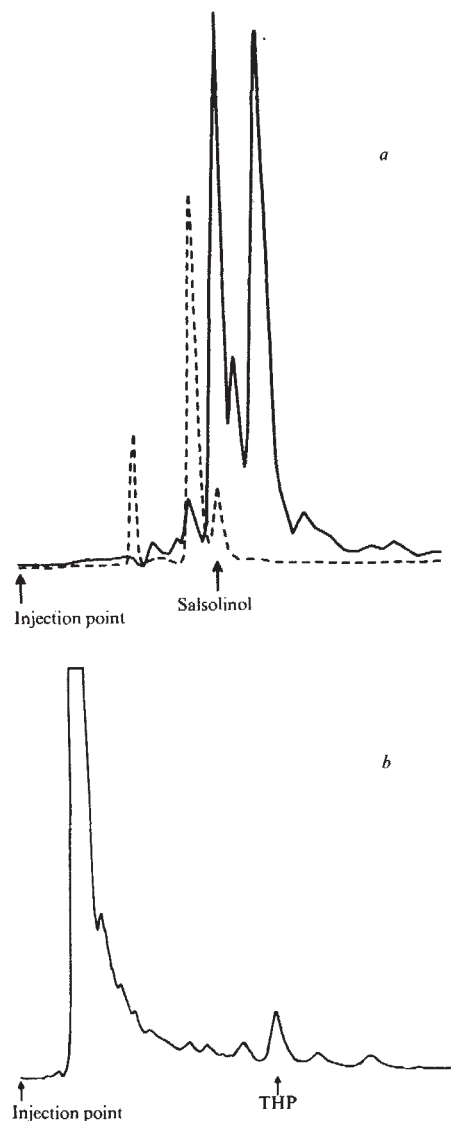


Fig. 4 Mass fragmentographic recordings of (a) m/e 617 (---) and m/e 602 (—) used to identify the tri-PFP derivative of salsolinol and (b) m/e 602 used to identify the penta-PFP derivative of tetrahydropapaveroline (THP) extracted from the urine of a parkinsonian patient during L-dopa therapy (Varian-MAT 311 by 3% OV1). The times of elution and, in the case of salsolinol, the ratio of the two ions, agreed with those of the PFP derivatives of authentic standards of salsolinol and tetrahydropapaveroline run on the same system.

rigidity and akinesia²³. There are indications that β -blockade with propranolol in affected patients on L-dopa treatment has a specific attenuating effect on tremor^{24,25}; thus it might conceivably act by countering the effect of the β -adrenergic agonist tetrahydropapaveroline, generated peripherally.

Whether tetrahydropapaveroline is taken up, stored and discharged from catecholamine binding sites in a similar

manner to the simpler tetrahydroisoquinolines⁴ is unknown. There has been much speculation, however, in two areas, largely based on the resemblance of its molecular structure to certain morphine-related alkaloids, particularly apomorphine (Fig. 5). It has been suggested that potentially addictive

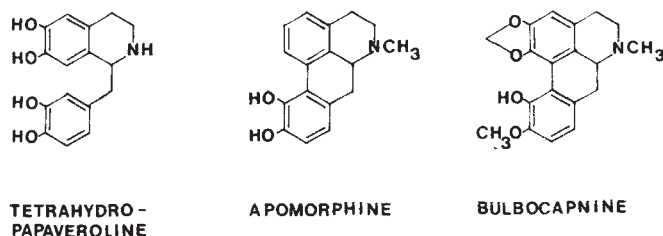


Fig. 5 Structures of the morphine-related alkaloids, apomorphine and bulbocapnine, showing their resemblance to tetrahydropapaveroline.

alkaloid derivatives of this type, derived from the interaction between biogenic amines and the ethanol metabolite, acetaldehyde, form the biochemical basis of ethanol addiction, although the resemblance between opiate and ethanol addiction is not very close (see ref. 4). Tetrahydropapaveroline, or a compound resembling it, has been suggested³ as the missing metabolite of L-dopa to account for some of the discrepancies associated with a "dopamine replacement" hypothesis of L-dopa action. Scheckel *et al.*²⁶ suggested that the unknown catecholamine-like material they detected in rat brains treated with dopa plus a peripheral decarboxylase inhibitor was a compound of this type, but their preliminary observations have never been followed up. In view of our results, it is obviously important to search for tetrahydropapaveroline in the brains of parkinsonian subjects who had received L-dopa. In this discussion, it is of particular interest to note that apomorphine itself leads to significant benefit in parkinsonism (see ref. 1). Unlike dopa, however, the most prominent early effect of which is the alleviation of akinesia, its most striking therapeutic action seems to be on tremor. Indeed, there is increasing evidence that L-dopa and apomorphine may have a synergistic action (e.g. ref. 27). In the light of our data, a working hypothesis that dopamine plus tetrahydropapaveroline and/or other related compounds in the Schiff base series generated at a slower rate is responsible for L-dopa action in parkinsonism seems credible. The question of whether the tetrahydroisoquinolines contribute, in addition, to normal brain function must also now be considered.

Schizophrenia

In his important theoretical paper, Sourkes³ drew attention to the similarity between the molecules of apomorphine and bulbocapnine (Fig. 5) and their possible relationship to a tetrahydropapaveroline-like precursor. Thus it is quite conceivable that bulbocapnine, which produces a catatonialike state in the experimental animal²⁸, may also act on central dopamine receptors in an opposite manner to that of dopamine and apomorphine²⁹. This experimental catatonia is somewhat reminiscent of one manifestation of schizophrenia; it is interesting, therefore, that L-dopa tends to bring about deterioration in schizophrenic subjects³⁰. Study of tetrahydroisoquinoline metabolism in schizophrenics might well be rewarding. It is even possible that another type of mental disorder, that associated with phenylketonuria, originates from the disproportionate presence of a carbonyl agent, phenylpyruvic acid, which may form a complex with a

biogenic amine such as dopamine¹². Our observations could lead to important new developments in neurological research.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Global Inventory and Distribution of Fallout Plutonium

PLUTONIUM isotopes, Pu-239,240 and Pu-238 in particular, have been injected into the stratosphere as a result of atmospheric nuclear weapons tests, and have reached the ground as particulate fallout. An accidental stratospheric injection of Pu-238 in 1964 resulted in almost a three-fold increase in the global fallout of this isotope, and we now describe the accumulated fallout and geographical distribution from this unexpected release as well as from weapons testing.

On April 21, 1964, a Transit navigational satellite was launched from Vandenberg Air Force Base in California. The payload included a Systems for Nuclear Auxiliary Power generator, SNAP-9A, containing 17 kCi or about 1 kg of Pu-238^{1,2}. Because the rocket failed to boost the satellite into orbital flight, the payload re-entered the atmosphere in the Southern Hemisphere^{2,3}.

Subsequent stratospheric checks⁴⁻⁶ indicated that the generator completely burned up during re-entry and turned into small particles at an altitude of about 50 km⁴. Concentrations of the SNAP Pu-238 in the stratosphere were measurable at the end of 1970 when it was estimated that less than a kCi remained above 12 km⁷. Attempts to measure the ground deposition rate on a continuous basis using a global fallout network were unsuccessful⁸. We finally used soil sampling to obtain integrated fallout samples, having previously demonstrated that soil at properly selected sites serves as a good indicator of cumulative fallout⁹.

The stratospheric inventories clearly indicated that by mid-1970, 95% of the SNAP Pu-238 was deposited on the Earth's surface. It was important to find out how it was distributed and to record the ground deposit. Knowledge of the fate of this debris is pertinent to studies of nuclear explosions in the upper atmosphere and the use of nuclear power sources in space.

In October 1970 until January 1971, 65 sites around the world were sampled by HASL personnel and scientists from other countries; soils were collected from undisturbed areas. Each sample consisted of ten 8.9 cm diameter cores taken to 30 cm depth, representing a surface area of 622 sq. cm. The measured Pu-238 included that from weapons testing plus the SNAP-9A contribution. The Pu-239,240 was assumed to be entirely derived from weapons testing.

In order to measure the total Pu-238 with a counting error (σ) of less than or equal to $\pm 10\%$, 1,000 g aliquots of soil were required. An acid leaching procedure¹⁰ used to accommodate this large size sample was found to be equally efficient¹¹ to complete dissolution.

The soil data are given in Table 1. The weapons Pu-238 contribution was obtained by multiplying the Pu-239,240 values by 0.024 (the average weapons Pu-238 to Pu-239,240 ratio found for six soils collected before fallout from the SNAP-9A). These soils were selected to cover a range of latitudes from 71° N to 35° S and the standard deviation of this average ratio was only $\pm 12\%$. The SNAP-9A Pu-238 is then simply the difference between the total measured Pu-238 and the weapons Pu-238.

The sites in Table 1 were grouped into ten degree latitude bands and the deposition values averaged. We plotted these averages against the sine of the latitude and made straight line extrapolations to zero at the poles. The average activities per square km in each ten degree latitude band are given in Table 2. The error term associated with the average for each ten degree latitude band is simply the standard deviation when two or more sites are located in the same band. Where only one site is represented or the value was derived by extrapolation, an average error for other bands in the hemisphere was applied.

The distribution pattern for weapons' plutonium shows heaviest deposition in the Northern Hemisphere temperate latitudes and a minimum in the equatorial region. There is a

Table 1 Pu Isotopes in Soil Collected 1970-71

Country	Site	Lat.°	Avg. annual precip. (cm)	mCi per km ²		
				Pu-239,240	Weapons	Pu-238 SNAP-9A
Northern Hemisphere						
Greenland	Thule	76.6	<15	0.33	0.008	(0.001)
USA	Barrow, Alaska	71.3	14	0.40	0.010	(0.004)
USA	Fairbanks, Alaska	64.8	28	0.85	0.020	0.020
Iceland	Reykjavik	64.1	86	1.5	0.036	0.016
USA	Palmer, Alaska	61.6	41	0.92	0.022	0.022
Norway	Bergen	60.4	192	3.1	0.074	0.049
Norway	Oslo	59.9	76	1.5	0.036	0.010
UK	Wick, Caith.	58.4	80	1.4	0.034	0.014
Denmark	Roskilde	55.6	59	1.2	0.029	0.009
UK	Wantage, Berks.	51.6	68	1.1	0.026	0.019
Germany	Munich	48.1	87	2.8	0.067	0.021
USA	Puyallup, Wash.	47.2	109	1.4	0.034	0.012
Italy	Ispra	45.8	145	2.5	0.060	0.025
USA	Orono, Maine	44.9	94	1.7	0.041	0.016
Japan	Sapporo	43.1	118	1.9	0.046	0.026
USA	Vermillion, S.D.	42.8	65	2.3	0.055	0.049
USA	Argonne, Ill.	41.8	84	2.1	0.050	0.019
USA	New York, N.Y.	40.8	90	2.6	0.062	0.035
USA	Salt Lake City, Ut.	40.8	40	2.6	0.062	0.032
USA	Denver, Colo.	39.8	38	1.8	0.043	0.034
USA	Manhattan, Kan.	39.2	77	2.4	0.058	0.029
Portugal	Sacavem	38.8	73	1.5	0.036	0.012
USA	Tulsa, Okla.	36.2	94	2.2	0.053	0.038
USA	Raleigh, N.C.	35.8	116	2.4	0.058	0.047
Japan	Tokyo	35.7	152	1.5	0.036	0.018
USA	Burbank, Calif.	34.2	26	0.73	0.018	0.003
Pakistan	Lahore	31.6	49	1.6	0.038	0.018
USA	Kingsville, Tex.	27.5	63	0.99	0.024	0.008
USA	Ft. Pierce, Fla.	27.5	142	1.0	0.024	0.010
USA	Weelaco, Tex.	26.2	61	0.88	0.021	0.016
USA	Papaikou, Hawaii	19.8	400	4.0	0.096	0.027
Venezuela	Maracay	10.3	96	0.24	0.006	0.003
Colombia	Bogota	4.6	106	0.13	0.003	(0.001)
Southern Hemisphere						
Kenya	Muguga	1.2	96	0.52	0.012	0.011
Brazil	Belém	1.5	268	0.42	0.010	0.019
Ecuador	Guayaquil	2.2	110	0.18	0.004	0.004
Angola	Luanda	8.8	43	0.093	0.002	0.006
Peru	Lima	12.1	4	0.12	0.003	0.015
Australia	Darwin	12.4	158	0.28	0.007	0.074
Mozambique	Nampula	15.2	102	0.15	0.004	0.026
Rhodesia	Salisbury	17.7	83	0.16	0.004	0.022
Australia	Townsville	19.2	119	0.18	0.004	0.046
Mozambique	Beira	19.8	151	0.17	0.004	0.036
Australia	Port Hedland	20.3	31	0.20	0.005	0.017
Brazil	Rio de Janeiro	22.9	150	0.61	0.015	0.148
Brazil	Angra dos Reis	23.0		0.60	0.014	0.098
Australia	Alice Springs	23.7	24	0.35	0.008	0.071
S. Africa	Pretoria	25.8	76	0.32	0.008	0.052
Mozambique	Lourenco Marques	26.0	80	0.25	0.006	0.043
Australia	Brisbane	27.4	108	0.40	0.010	0.061
Australia	Perth	33.1	102	0.41	0.010	0.056
Chile	Santiago	33.5	36	0.22	0.005	0.043
S. Africa	Stellenbosch	33.9	70	0.26	0.006	0.031
Australia	Sydney	34.8	144	0.47	0.011	0.050
Argentina	Buenos Aires	34.8	99	0.49	0.012	0.081
Australia	Adelaide	35.2	88	0.33	0.008	0.078
New Zealand	N. Auckland	35.8	181	0.43	0.010	0.086
Australia	Melbourne	38.2	103	0.55	0.013	0.065
Chile	Puerto Montt	41.5	260	0.20	0.005	0.057
Australia	Hobart	41.5	83	0.26	0.006	0.045
New Zealand	Greymouth	42.5	250	0.67	0.016	0.126
New Zealand	S. Canterbury	44.4	57	0.28	0.007	0.048
Chile	Punta Arenas	53.2	22	(0.19)	(0.004)	(0.034)

Table 2 Average Latitudinal Distributions of Cumulative Pu-239,240 and Pu-238 Fallout

Hemi-sphere	Lat. band°	mCi per km ²		
		Pu-239,240	Weapons Pu-238	SNAP-9A Pu-238
Northern	90-80	(0.10 ± 0.04)	(0.002 ± 0.001)	(< 0.001)
	80-70	0.36 ± 0.05	0.009 ± 0.001	< 0.001
	70-60	1.6 ± 1.0	0.038 ± 0.025	0.026 ± 0.015
	60-50	1.3 ± 0.2	0.031 ± 0.004	0.013 ± 0.004
	50-40	2.2 ± 0.5	0.053 ± 0.011	0.026 ± 0.011
	40-30	1.8 ± 0.6	0.042 ± 0.014	0.025 ± 0.015
	30-20	0.96 ± 0.07	0.023 ± 0.002	0.011 ± 0.004
	20-10	0.24 ± 0.10	0.006 ± 0.002	0.003 ± 0.002
	10-0	0.13 ± 0.06	0.003 ± 0.001	< 0.001
Southern	0-10	0.30 ± 0.20	0.007 ± 0.005	0.010 ± 0.007
	10-20	0.18 ± 0.05	0.004 ± 0.001	0.036 ± 0.021
	20-30	0.39 ± 0.16	0.009 ± 0.004	0.070 ± 0.042
	30-40	0.40 ± 0.12	0.009 ± 0.003	0.061 ± 0.020
	40-50	0.35 ± 0.21	0.008 ± 0.005	0.069 ± 0.038
	50-60	(0.20 ± 0.09)	(0.005 ± 0.002)	(0.044 ± 0.023)
	60-70	(0.10 ± 0.04)	(0.002 ± 0.001)	(0.022 ± 0.012)
	70-80	(0.03 ± 0.01)	(0.001 ± 0.001)	(0.008 ± 0.005)
	80-90	(0.01 ± 0.004)	(< 0.001)	(0.004 ± 0.002)

Results in parentheses were derived by extrapolation; error terms are standard deviations.

slight rise in the Southern Hemisphere temperate zone which, at its peak, is about one-fifth of the Northern Hemisphere maximum. The SNAP-9A Pu-238 has an entirely different distribution pattern. Most of the SNAP debris has deposited in the Southern Hemisphere where the maximum fallout is 2.5 times that in the Northern Hemisphere.

Table 3 Inventory of Pu-239,240 and Pu-238 Fallout

	kCi deposited		
	Pu-239,240	Weapons Pu-238	SNAP-9A Pu-238
Northern Hemisphere	256 ± 33	6.1 ± 0.8	3.1 ± 0.8
Southern Hemisphere	69 ± 14	1.6 ± 0.3	10.8 ± 2.1
Global	325 ± 36	7.7 ± 0.9	13.9 ± 2.2

The average and extrapolated activities per square km for each ten degree latitude band were multiplied by the latitude band area to calculate the total deposit in kCi. These values were then summed to obtain the inventories in Table 3. The errors were propagated by conventional means.

The total Pu-239 deposit was 326 ± 36 kCi and the weapons Pu-238, 7.7 ± 0.9 kCi. Our estimate of the SNAP-9A Pu-238 deposit, 13.9 ± 2.2 kCi, is in reasonable agreement with the 16 kCi we would expect to have fallen out based on the stratospheric inventory. Over 75% of the total SNAP Pu-238 deposit is in the Southern Hemisphere whereas only about 20% of the total weapons Pu-238 fallout occurred in that hemisphere. This accidental release of Pu-238 almost tripled the global deposit of this plutonium isotope by 1970. The distributions and inventories of the weapons Pu-238 and Pu-239,240 are in reasonable agreement with estimates based on Sr-90 deposition and Pu-239,240 to Sr-90 ratio measurements¹².

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Electron Microscopy of Monomolecular Layers of Thorium Atoms

IMAGES of heavy atoms in single molecules have recently been obtained with scanning transmission electron microscopes as well as conventional electron microscopes in both bright and dark-field operation¹⁻⁴. Because of the random orientation of isolated molecules on the specimen support, only a few of them show the true interatomic distances in parallel projection. Also molecules could stack themselves during preparation. Finally, the limited depth of focus of electron microscopes at 100 kV when viewing atoms⁵ makes it necessary to obtain a flat specimen.

To overcome these difficulties it is necessary to prepare monomolecular films 6-30 Å thick. Such monolayers of atoms can be formed on liquid-air interfaces in Langmuir troughs and it is possible to obtain different molecular structures with the molecules tagged with heavy atoms. For electron micro-

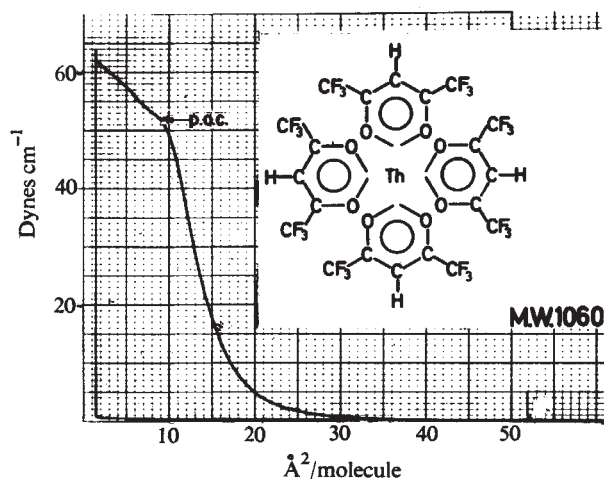


Fig. 1 Isotherm of thorium-hexafluoroacetylacetonate at 3°C. p.o.c., Point of collapse.

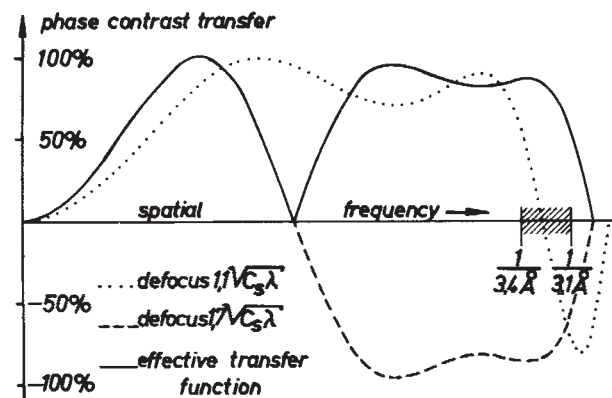


Fig. 2 Phase contrast transfer functions for $C_s = 1.32$ mm, $C_e = 1.62$ mm, $\lambda = 0.037$ Å (100 kV) and Maxwellian velocity distribution at $T = 2,900$ K.

scopy (EM) test specimens with single heavy atom patterns of controllable spatial frequency can be obtained. Discrimination between elements⁶ can be tested using arrays of atoms with different atomic numbers and so any damage caused by irradiation should be detected directly.

Monolayers or bilayers are of considerable biological relevance as they are similar to the molecular arrangement in cell membranes and may provide structural information on molecular interactions at interfaces.

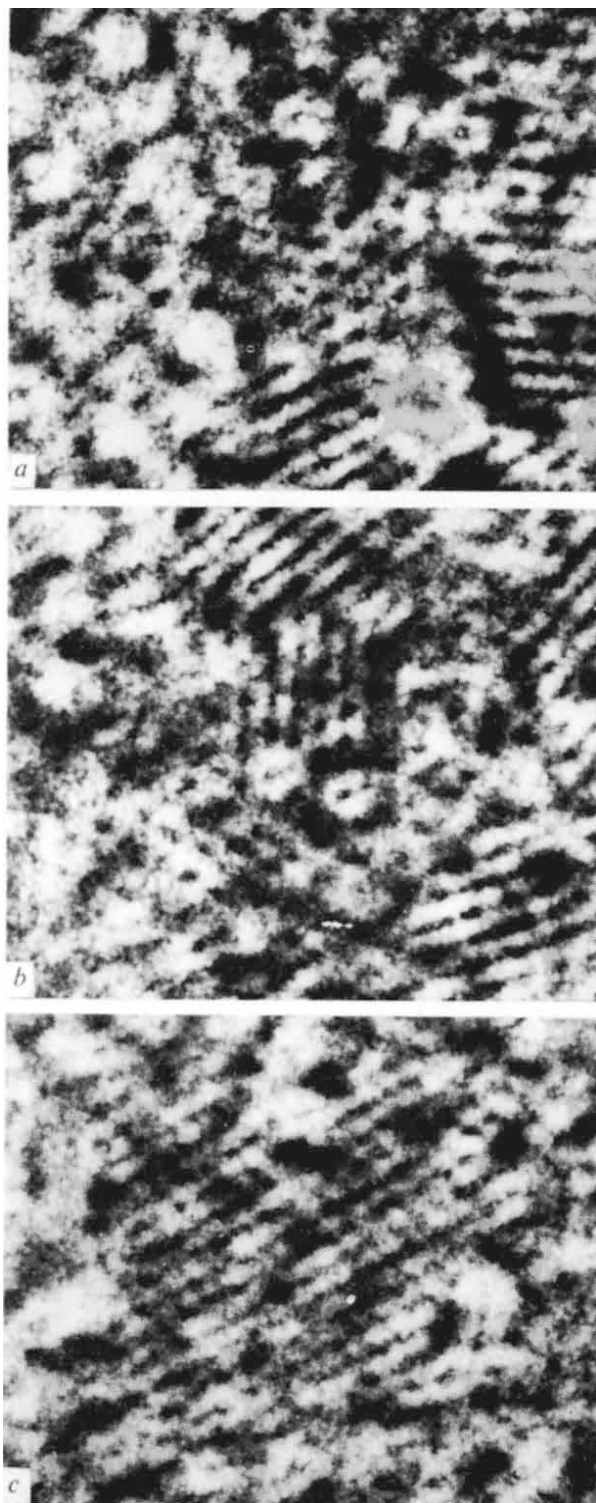


Fig. 3 Thorium atom patterns of monomolecular film of thorium-hexafluoroacetylacetonate on a support of thin carbon. Electron micrographs: 4.3×10^5 magnification; prints at magnification of 10^7 .

We used a specially designed Langmuir trough to generate monomolecular films and to transfer them to specimen supports for investigation by an electron microscope. A pressure-sensing float and a barrier driven by a motor, each fencing the film and connected to a recorder *via* amplifiers and interconnected by a feedback-loop, made it possible, first, to obtain isotherms of films to survey their performance and, second, to maintain any selected pressure of the film during transfer to the specimen support⁷.

Preliminary transfer experiments showed the wettability of supports used for specimens and radiation damage to be critical factors⁸. To prove the feasibility of electron-optical imaging of heavy atom patterns we transferred a monolayer of thorium-hexafluoroacetylacetonate to a carbon film—thus combining a high atomic number element with a structure of low radiation sensitivity on a sufficiently wettable support. The isotherm for 3° C (Fig. 1) reaches the point of collapse at 52 dyne cm^{-1} and a mean area of 9 Å² per molecule. We transferred this monolayer at 32 dyne cm^{-1} and expected interatomic distances between 3.1 Å and 3.3 Å in a hexagonal array judging from the Archimedean square antiprismatic structure of the molecule⁹.

Electron micrographs were obtained at 100 kV with a Siemens Elmiskop 101 equipped with special objective lens (spherical aberration $C_s = 1.32$ mm; chromatic aberration $C_c = 1.62$ mm) and improved stability of lens current and high tension supplies.

The chief source of contrast in high resolution electron microscopy is the interference of the diffracted wave, whose phase is changed by the field of the atomic nucleus, with the undiffracted wave. Therefore spatially coherent illumination is used and the properties of the objective are given by coherent phase transfer functions dependent on spherical aberration and defocus. Obviously the expected spatial frequencies around 3.2 Å⁻¹ would not be properly imaged at the commonly used Scherzer defocus $1.1 \times \sqrt{C_s \lambda}$ (ref. 10) because of the zero crossing of the respective transfer function (see Fig. 2). Therefore we used the second main transfer interval¹¹ at a defocus of $1.7 \sqrt{C_s \lambda}$ which, of course, makes the "atom"-dot in the picture to be bright. To restore readability of the images and to improve resolution and contrast, we processed our micrographs by spatial filtering of the Maréchal type¹², which effectively reverses the negative lobe of the transfer function. Fig. 3 shows prints of the processed micrographs taken at 435,000 times the primary magnification enlarged to 10^7 magnification. Small areas of undisturbed monolayer with single heavy atom pattern can be recognized. As the surface of the supporting carbon film is not smooth, the monolayer is obviously broken up into many small panels. If these are not nearly perpendicular to the optical axis, their frequencies are raised by tilted projection into the zero-crossing region of the transfer function. Therefore some atom rows do not appear as single dots.

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Migration of Trace Metals in Snow

SEVERAL workers¹⁻⁴ have established that elemental mercury vapour is present in soils overlying mineralized bedrock, and the vapour can be used in prospecting for base metal sulphide deposits⁵. It is therefore possible that mercury vapour will emanate from soils into overlying snows and that the trace metals in fallen snow can be used to indicate the presence of underlying, or at least nearby, mineralization. Kolotov and colleagues⁶ have already reported the presence of such dispersion aureoles for zinc, copper and lead, as total heavy metals, in snow above a deposit of tin ore, but they ascribe the phenomenon to falling snow carrying down dust clouds which were thought to exist over the deposit.

by atomic absorption spectrometry after extraction with methyl iso-butyl ketone and APDC. Mn, Na, K, Fe and Hg were similarly determined but without previous extraction.

In January 1971, fresh fallen snow samples collected in Ottawa were shown to contain 3.5 to 4.0 p.p.b. Hg (parts per thousand million) with a mean of 3.7 p.p.b. The snow contained scattered soot particles which are thought to be residues of burned fuel-oil and gasoline. The elevated Hg values probably reflect the low, but in this case significant, Hg content of fossil fuels⁷.

In January 1972, snow samples from the same area unaccountably showed much less Hg but did contain a variety of other metals. Analytical data are presented in Table 1.

I consider that the prime influence on these metal contents is the proximity of a 6-lane freeway. Sample 3 was collected closest to the road (100 yards). The others were further removed (500 yards). Roads in the Ottawa area are salted during winter snowfalls causing heavy traffic to raise a mist of melt water into the atmosphere above them. Elevated contents of Na, Zn, Pb, Ni and Fe in nearby fallen snow clearly support the contention that falling snow is an efficient scavenger of airborne dust and aerosols.

Table 1 Trace Metal Contents of Snow from an Urban Area

Sample No.	Hg (p.p.b.)	Cu (p.p.b.)	Zn (p.p.b.)	Pb (p.p.b.)	Ni (p.p.b.)	Cd (p.p.b.)	Na (p.p.m.)	K (p.p.m.)	Fe (p.p.b.)
1	0.065	17	22	87	21	0.3	17	1.2	90
2	0.071	15	18	55	20	<0.2	32	2.5	30
3	0.123	21	192	410	17	1.0	328	0.9	130
4	0.071	69	27	78	10	0.7	3	0.4	—

Various mechanisms by which metals may be trapped in snow have been tested by taking samples in three types of environment—urban, unmineralized rural and mineralized rural. Snows were sampled in the city of Ottawa and in the vicinity of a mercury prospect near Clyde Forks in Eastern Ontario. At the latter site, mineralization consists of veinlets of chalcopyrite, tetrahedrite and pyrite with which are associated the metals, copper, mercury, lead, zinc and manganese. The same metals are also dispersed to some extent in overlying soils. Snow was collected over these soils and also in areas remote from mineralization.

The analytical data presented represent whole snow samples. Melt water was coarsely filtered ('Whatman 30') to remove gross contaminants. Cu, Zn, Pb, Cd and Ni were determined

For rural areas the data in Table 2 show that the metal contents of fresh surface snow in unmineralized areas are much less than in Ottawa. These figures probably represent normal background levels in the atmosphere. However, snow strata collected in mineralized zones contain significant Hg, Pb, Zn, Cu and Mn (Table 2) particularly in sub-surface samples near to the soils. I considered that these and probably other metals readily migrate upwards into the snow strata from underlying mineralized soils to reach the levels indicated in less than 2.5 months. Significant concentration gradients are observed only for the major ore metals, Hg and Cu, where 40 to 50-fold differences in metal content are observed across a 5 foot depth of snow. Gradients measured for the minor ore metals, Zn, Pb and Mn, are by comparison quite low and similar to those measured at an unmineralized site. They therefore represent migration from soils with common background contents of these metals (Table 3).

Table 2 Trace Metals in Snow

Snow horizon depth (in feet) from snow surface		Hg	Cu	Zn	Pb	Mn
Samples from an unmineralized site						
Surface	0-1	<0.01	3	8	5	<15
Middle	1-2	<0.01	4	15	15	<15
Lower	3-4.5	<0.01	8	16	12	<15
Total depth, 5 feet						
Samples from a mineralized site						
Surface	0-1	<0.01	3	12	9	<15
	1-2	0.40	27	28	11	<15
Middle	2-3	0.19	73	14	10	<15
	3-4	0.30	74	182	2	15
Lower	4-5	0.52	98	18	4	20
Total depth, 5 feet						

All values in p.p.b. (parts per thousand million).

Table 3 Trace Metals in Soils

Soil description	Hg	Cu	Zn	Pb	Mn
Humic soil overlying mineralization	278	370	65	12	910
Mineralized gravels	1,200	11,800	400	20	1,030
Humic soil from an unmineralized zone	0.38	31	220	55	1,600
Mineralization is covered by 1 to 2 feet of soils					

All values in p.p.m.

The migration mechanism in this case is probably related to the upward movement of pellicular waters which carry metal ions and perhaps humate complexes from the soils into the overlying snow strata. Mercury may also migrate as dissolved elemental vapour.

It is worth noting that the trace metal levels found in snow in each situation are comparable. The presence of elevated metal levels may therefore be regarded as man-made contamination in the first case and as natural contamination in the second case.

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Thermal Radiation from a Tropospheric Dust Suspension

INCREASING concern is being felt by scientists and laymen alike over the possibility of climate alteration due to increased atmospheric dust. Although the early consensus of most scientists was that increasing the dust content of the atmosphere would tend to cool the Earth, owing to an increased backscatter to space of solar radiation, several investigators have recently published studies indicating that this conclusion may not unconditionally hold true¹⁻³. Thus, the question of whether

increased atmospheric aerosol would tend to warm or cool the Earth, *via* its interaction with solar radiation, remains an open one, awaiting additional data on the solar scattering and absorptive properties of natural and man-made aerosols.

A second approach to the question, however, is to consider the interaction of atmospheric aerosol with long-wave terrestrial radiation. Although practically all previous studies considered this interaction to be negligible^{3,4}, there have been some recent experimental indications that quite the opposite may be true⁵⁻⁷; and in this paper I report on one additional example.

On the evening of July 29, 1972, a huge but weak dust storm hit Phoenix, Arizona, at about 2000 MST (Mountain Standard Time), and shortly thereafter exhausted itself over the metropolitan area. The finer residue of the dust remained in suspension through most of July 30, reducing visibilities from 52 to 8 km. Dust storms of this type at Phoenix generally raise a surface dust cloud that extends to a height of 2,500 m (ref. 8); and the dust suspension of July 30 was estimated to extend to at least this height, which is also the level of the mean maximum mixing height at that time of year⁹.

July 30 was a cloudless day, and atmospheric thermal radiation measurements were collected from 1000 MST until 1500 MST, when the dust began to move out of the instrument area. These data were obtained by subtracting the solar radiation measured by a temperature compensated Eppley pyranometer from the mean hemispherical all-wave radiation obtained from three Fritschen-type net radiometers^{10,11} converted to hemispherical operation by the technique of Idso^{12,13}. The results are shown in Table 1 together with measurements on three other adjacent cloudless days, screen-level air temperature and vapour pressure, and mean wind speed and visibility. The visibility data give an indication of the relative clarity of the three normal cloudless days as compared to July 30; and the wind speed data show the potential of all days for similar temperature profiles in the boundary layer.

Using the Brunt equation¹⁴ to estimate the effects on the atmospheric thermal radiation of the reduced air temperature but increased vapour pressure of July 30 compared with the

Table 1 Air Temperature, Vapour Pressure, Visibility, Wind Speed and Atmospheric Thermal Radiation on Three Normal Clear Days (July 27, 31; August 1) and the Day of the Dust Suspension (July 30)

Parameter	Time	1000	1030	1100	1130	1200	1230	1300	1330	1400	1430	1500	Av.
Air temperature (°C)	July 27	37.2	38.0	38.2	41.1	39.7	40.8	43.8	45.7	44.8	44.3	43.4	41.5
	July 31	39.1	39.4	44.8	44.3	43.8	45.0	45.0	45.9	44.6	43.4	45.9	43.7
	August 1	39.4	42.9	43.8	43.6	44.0	44.8	45.5	46.6	45.7	48.0	45.5	44.5
	Average	38.5	40.1	42.3	43.0	42.5	43.5	44.8	46.1	45.0	45.2	44.9	43.2
	July 30	38.2	40.3	39.7	41.1	41.9	42.9	42.2	41.9	43.4	43.4	44.6	41.8
Vapour pressure (mbar)	July 27	18.4	19.2	18.1	21.1	16.0	16.9	15.3	17.0	15.2	14.8	14.1	16.9
	July 31	15.5	15.7	19.9	19.4	17.1	18.2	16.3	17.1	15.0	14.1	14.1	16.6
	August 1	12.9	15.5	15.3	15.2	13.6	14.2	12.8	13.5	13.9	15.6	12.8	14.1
	Average	15.6	16.8	17.8	18.6	15.6	16.4	14.8	15.9	14.7	14.8	13.7	15.9
	July 30	17.4	19.5	18.2	19.6	19.6	20.6	18.2	18.0	18.5	18.5	17.8	18.7
Visibility (km)	July 27	48		48		48		48		48		56	49
	July 31	48		48		48		48		48		48	48
	August 1	56		56		56		56		64		64	59
	Average	51		51		51		51		53		56	52
	July 30	8		6		8		8		8		10	8
Wind speed (m s ⁻¹)	July 27	1.0		2.1		3.1		3.6		5.1		2.6	2.9
	July 31	1.5		3.1		2.6		3.6		5.7		3.6	3.4
	August 1	3.6		2.1		2.6		4.6		4.6		4.6	3.7
	Average	2.0		2.4		2.8		3.9		5.1		3.6	3.3
	July 30	1.5		5.1		3.6		4.1		4.1		5.7	4.0
Thermal radiation (cal cm ⁻² min ⁻¹)	July 27	0.762	0.756	0.757	0.746	0.760	0.748	0.770	0.777	0.772	0.772	0.750	0.761
	July 31	0.766	0.779	0.706	0.732	0.771	0.774	0.791	0.776	0.774	0.774	0.761	0.764
	August 1	0.736	0.784	0.737	0.760	0.765	0.772	0.774	0.762	0.776	0.771	0.761	0.763
	Average	0.755	0.773	0.733	0.746	0.765	0.765	0.778	0.772	0.774	0.772	0.757	0.763
	July 30	0.778	0.786	0.814	0.816	0.814	0.827	0.825	0.820	0.806	0.809	0.791	0.808

other three cloudless days, it was calculated that this energy flux should have been 1.6% greater on July 30. The actual measurements showed it to be 5.9% greater, however, implying that the dust suspension of July 30 was responsible for a 4.3% increase in atmospheric thermal radiation.

That this figure is probably a very conservative estimate of the potential long-wave aerosol effects may be shown by use of the concept of effective atmospheric emittance, defined as incoming thermal radiation received per calculated blackbody radiation at measured screen level air temperature. For the three normal cloudless days the mean effective atmospheric emittance over the 1000–1500 period was 0.955. As 1.000 represents the theoretical upper limit of this parameter, it is seen that dust effects could only have increased it by 4.7%. The measured value of 4.3% differs from this potential value by an amount that is of the order of the resolution of the instrumentation employed in obtaining it. Thus, it may be concluded that the dust suspension of July 30 transformed the atmosphere into an effective blackbody, limiting any further increases in the atmospheric thermal radiation and prohibiting the experimental determination of its full potential for long-wave radiation interaction.

This point is significant in that the effective atmospheric emittance is temperature dependent, and the mean value for the Earth as a whole is much lower than that prevailing at Phoenix on the days of this study. In particular, the effective atmospheric emittance appears to have a minimum value of about 0.74 in the region of 0° C, tending towards unity at both higher and lower temperatures¹⁵ to yield a mean value of about 0.88¹⁶. Thus, the long-wave effects of an atmospheric aerosol could be much greater than the 4.3% increase they effected in the atmospheric thermal radiation in this study. Indeed, measurements I made during a winter dust storm indicated that an increase in atmospheric thermal radiation of 12 to 13% may be brought about by low level dust⁷.

Notwithstanding this greater potential effect, just the basic 4.3% increase in effective atmospheric emittance discovered in this study is sufficient to alter vastly the Earth's climate through the so-called "greenhouse effect". Via this well-known mechanism, any increase in the emissivity of the Earth's atmosphere will produce an incremental temperature rise near the planet's surface. Using the heat balance model of Haltiner and Martin¹⁶ for mean Earth conditions, it may be calculated that the long-wave aerosol effects detected in this study could conceivably warm the Earth by 3 or 4° C. In view of the uncertainty associated with even the qualitative effects of aerosol interaction with solar radiation, these results suggest that the likely effect of increasing the dust content of the atmosphere would be to produce a warming trend at the Earth's surface.

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Tritium Contamination of Deuterium Compounds

Two reports^{1,2} have appeared which indicate that various commercial deuterated reagents and solvents contain appreciable quantities of tritium. Because this matter is of concern especially in view of the wide use of deuterated compounds as solvents for n.m.r. spectroscopy I wish to report that these observations are in error. Leete¹ indicated that only the deuterium oxide (D₂O) supplied by one company was low in tritium. In fact D₂O supplied by that company before the middle of 1971 was, at times, also high in tritium, and since then D₂O supplied by most companies has been low in tritium. The US Atomic Energy Commission (AEC) is the prime source of D₂O to nearly all suppliers and the results shown in Table 1 illustrate the dramatic reduction in tritium content of recent samples of D₂O obtained from the AEC.

Table 1 Tritium Content of D₂O Received from AEC

Date	μCi ml. ⁻¹ of D ₂ O
September, 1969	6.0
November, 1969	6.0
April, 1970	6.0
April, 1970	0.06
May, 1970	0.12
July, 1970	0.09
September, 1970	0.06
September, 1970	0.09
November, 1970	0.1
December, 1970	0.27
January, 1971	0.02
March, 1971	0.0012
July, 1971	0.00047
October, 1971	0.0004
January, 1972	0.0003
March, 1972	0.00031
March, 1972	0.00008
May, 1972	0.0004
August, 1972	0.00008

Thus, Leete's results (private communication) are no longer valid, having been obtained from old samples of D₂O; the heavy water currently available contains very little tritium. Because D₂O is the prime source of deuterium for other deuterated materials the reduction in the tritium content of D₂O will result in a marked decrease in the tritium content of other deuterated chemicals.

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Micro-fission Explosions and Controlled Release of Thermonuclear Energy

THE possibility of igniting a thermonuclear micro-explosion by a laser pulse has recently attracted great interest as a result of the proposal to lower the energy requirements by imploding a T-D thermonuclear pellet with a laser pulse of proper shape,

thereby increasing its density by a factor of $\sim 10^4$ over the solid density¹. If this concept of high density compression is applied to a fissionable pellet, consisting of ^{235}U , ^{233}U or ^{239}Pu , very small critical masses become possible. These critical masses can be further reduced by surrounding the fissionable pellet with a neutron reflecting material to be compressed, together with the pellet, to high densities. Finally, if this neutron reflecting material consists of thermonuclear material such as T-D, the fission chain reaction rising in the critical pellet will ignite a small thermonuclear explosion in the neutron reflector. The fission chain reaction rises at a much faster rate than for solid densities (such as in atomic bombs) because of the greatly increased densities. The release of thermonuclear neutrons in the reflector will accelerate the fission chain reaction to an even higher speed whereby the heating of the thermonuclear material is accelerated in return, resulting in even more thermonuclear neutrons.

The system may be useful as a small fission power plant, especially for a safe breeder reactor or as a combined fission-fusion power plant. Another important application of this concept is for space propulsion.

In the spherical ablation implosion concept of laser fusion, pressures up to $p \approx 10^{18}$ dyne cm^{-2} are predicted. If these pressures are applied to a solid such as ^{235}U , ^{233}U or ^{239}Pu the density can be calculated from a Thomas-Fermi equation of state. This implies densities ~ 240 times larger than solid densities with an atomic number density of $N \approx 1.17 \times 10^{25}$ cm^{-3} .

The critical radius for a spherical mass of fissionable material is²

$$R_0 = \frac{\pi}{B_m} - d \quad (1)$$

with $B_m^2 = 3\sigma_s\sigma_f N^2 (v-1)$, $d \approx 0.71/N\sigma_s$, where $\sigma_s \approx 2 \times 10^{-24}$ cm^2 , $\sigma_f \approx 2 \times 10^{-24}$ cm^2 are the neutron cross-sections for scattering and fission, and $v \approx 2.9$ the neutron multiplication factor. From equation (1) $R_0 \approx 2.6 \times 10^{-2}$ cm, with a pellet volume of $V \approx 7.35 \times 10^{-3}$ cm^3 . The critical mass at 240 times solid density is then $m \approx 0.34$ gm.

The compression energy is given by $E \approx (2/3)pV \approx 4.9 \times 10^{13}$ erg = 4.9 MJ. The total energy required to reach this highly compressed state will be larger because a substantial portion of the energy will go into the ablation products. Nevertheless it is clear that the energies needed for compression are here in the same order of magnitude range as for the requirement to ignite a T-D micro-fusion explosion. Further, relativistic electron beams are already available at energies in this range and they may be similarly employed for compression; they have already been proposed as an alternative to laser fusion ignition systems³.

For a spherical pellet surrounded by a strong neutron reflector the critical radius is²

$$R = \frac{3}{2} \frac{D_r}{D_c B_m^2 T} \left[1 + \left(1 + \frac{4}{3} \frac{D_c}{D_r} B_m^2 T^2 \right)^{1/2} \right] \quad (2)$$

where $D_c \approx 3/N_c\sigma_{cs}$, $D_r \approx 3/N_r\sigma_{rs}$ are the neutron diffusion coefficients in the fissionable core (D_c) and neutron reflector (D_r), σ_{cs} and σ_{rs} are the neutron scattering cross-sections in the core and reflector. If the reflector consists of T-D the atomic number density at $p = 10^{18}$ dyne cm^{-2} is $N_r \approx 5 \times 10^{26}$ cm^{-3} . With $\sigma_{rs} \approx 2 \times 10^{-24}$ cm^2 and for $R \approx T$, equation (2) gives $R \approx 4.7 \times 10^{-3}$ cm with a critical mass of $m \approx 1.9 \times 10^{-3}$ gm. The compression energy in this case is $E \approx (2/3)pV \approx 2.3 \times 10^5$ J with $V = (4\pi/3)(R+T)^3$.

The fission chain reaction proceeds according to the equation

$$n = n_0 \exp(N\sigma_f v_0 (v-1)t) \quad (3)$$

where n is the number of fission neutrons released at time t and $v_0 \approx 10^9$ cm s^{-1} is the neutron velocity. For the numbers given above.

$$n \approx n_0 \exp(4.7 \times 10^{10} t) \quad (4)$$

From equation (4) the e-folding time $t_e \approx 2 \times 10^{-11}$ s. The hydrodynamic disassembly time at keV temperatures is given by $\tau \approx R/v \sim 10^{-9}$ s. The chain reaction will have progressed during this inertial confinement time to $n \approx n_0 e^{50} \sim 10^{22} n_0$. This very large number of neutrons delivered indicates that a very high fission yield occurs, substantially larger than that which can be achieved in atomic bombs.

In order to obtain a fast rising chain reaction the pellet must be somewhat larger than the critical dimension which in turn requires a somewhat larger energy input for compression than estimated above.

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Mercury in Lake Sediments: a Possible Indicator of Technological Growth

GOLDBERG¹ has estimated that at present man is responsible for about half of the total Hg released into the environment, and pollutant Hg has already been found in snow and ice² and near-shore marine sediments³. It seemed to us that lake sediments, which geologically have a relatively rapid rate of deposition, might produce further evidence of man's input of Hg to the environment. This has been shown to occur in sediments from a Canadian lake which receives industrial waste⁴. Our sediments are from an English lake in a predominantly rural area.

A 1 m undisturbed sediment core was obtained from the South Basin, Lake Windermere, England, using a pneumatic corer⁵. There was a transition in the sediment at ~ 28 cm depth; above this the sediments consisted of poorly compacted black *Asterionella* muds, and below it was a sequence of well compacted brown clays. Hg in the sediments was determined by a modification of a cold vapour atomic absorption procedure⁶. For the top ~ 28 cm of the core (black mud) the accumulation rate (2.7 mm yr^{-1}) of wet sediment was determined by a ^{210}Pb radionuclide technique adjusted to take account of the enhanced water content of the topmost 10 cm, and for the underlying brown clay the value given by Creer *et al.*¹⁰ (average 0.35 mm yr^{-1}) was used. There is a stepwise increase in Hg content upwards within the core (Table 1).

Methylation of inorganic Hg by organisms can result in the release, upward movement and subsequent recycling of a volatile form of mercury. But, in the presence of methogenic microorganisms methylation is confined to the upper ~ 3 cm of sediment⁸, and occurs at the rate of $\sim 0.1\%$ per year of the total Hg present. Because the sedimentation rate at the site of the present core is ~ 2.7 mm yr^{-1} a sediment cover of 3 cm will be supplied in ~ 12 yr and the loss of Hg from the layer of active methylation will be negligible and cannot account for the increase in Hg which occurs at various levels in the core.

The upward post-depositional migration of inorganic Hg through the interstitial waters by ionic or molecular diffusion, or sediment compaction, could lead to an enhancement of Hg in the upper portions of the core. On present evidence this process is difficult to assess. Thomas⁴ rejects Hg mobility as a controlling factor in the distribution of Hg in a Canadian lake core. Further, evidence⁹ from areas where the discharge of Hg has occurred, but has been halted, suggests that there has been little movement of Hg within sediments over periods of 15 to

Table 1 Mercury Content of Core Samples

Depth in core (cm)	Date	Hg content (p.p.b.)	Group
5	1950	1,050	Group D;
10	1935	1,034	average
15	1915	1,005	1,026 p.p.b.
20	1895	620	Group C;
24	1880	579	average
26	1870	626	608 p.p.b.
30	1800	363	Group B;
34	1670	175	average
40	1520	331	286 p.p.b.
44	1400	280	
50	1260	152	Group A;
60	1020	95	average
70	750	90	122 p.p.b.
74	660	168	
78	520	105	

45 yr. But the possibility of post-depositional movement of Hg within a sediment profile cannot be wholly excluded.

The vertical distribution of Hg could result from an increasing supply of Hg over the past half millenium, probably due to an enhanced release of Hg by man's activities. These include: denudation of land surfaces; heavy industry, mining and quarrying; burning of fossil fuels; and sewage disposal. Much of the Hg thus released initially enters the atmosphere, although some is introduced directly into natural waters. We suggest that the Hg contents of the core reflect, at least in part, this input by man. The sediments of Group A (Table 1) have an average Hg content of 122 p.p.b. which probably represents the local "background". Since ~1400 AD there has been a stepwise increase in Hg contents of the sediments which may correlate with man's activities, for example the change from Group B to C may reflect the onset of the Industrial Revolution. Pennington⁷ suggests that the change from the brown clay to the black *Asterionella* ooze was caused by an increase in dissolved salts due to sewage pollution following human settlement on the lake shore. It is likely that the introduction of sewage in recent years will have resulted in an increased supply of Hg in the lake⁸. The combination of various factors comprising technological growth has, therefore, resulted in a progressive increase of Hg in the sediments of Lake Windermere.

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BIOLOGICAL SCIENCES

Molecular Model for Phase Transition in Biological Membranes

STUDY of phase transition of lipid molecules in biological membranes has been stimulated by the possible significance of this transition for membrane function, particularly for diffusion across the membrane. Here I propose for the first time a simple molecular field model which provides a quantitative measure of the ordering of membrane lipids as a function of temperature. It is based on theories which describe ordering in thermotropic liquid crystals, as described by McMillan¹.

In bimolecular lipid layers of artificial and natural membranes, the basic units are not rigid molecules as in classical liquid crystals but elements of hydrocarbon chains. The orientation of single elements in these is restricted because the valence bond angle remains constant and only a fixed number of *gauche* configurations of the molecule form energetically accessible states. Such a chain is statistically equivalent to a chain composed of a smaller number of elements with no restrictions to the possible orientations². Here I shall treat the hydrocarbon chain of the lipid molecule as consisting of a number of segments which can assume arbitrary orientation. The number of independent segments for a given molecule is determined by comparison with the experiment described below.

The dispersive or Van der Waals forces lead to an anisotropic interaction of chain segments. This interaction can be described by the liquid crystal model of Maier and Saupe

$$V_{12} = - \frac{V_0}{N} \left(\frac{3}{2} \cos^2 \theta_{12} - \frac{1}{2} \right)$$

where θ_{12} is the angle between the long axes and N the density of the molecules. In the molecular field approximation, this interaction leads to the one-particle potential in the molecular field η :

$$V_1(\eta) = - V_0 \left(\frac{3}{2} \cos^2 \theta - \frac{1}{2} \right) \eta$$

$$\eta = \left\langle \frac{3}{2} \cos^2 \theta - \frac{1}{2} \right\rangle$$

where θ is the angle between the long axis of the segment and the preferred axis of orientation.

Further terms in the one-particle potential derive from other factors influencing the molecular order. In natural membranes, molecules packed between the hydrocarbon chains act to restrict the freedom of orientation and impose a degree of order on the chains. Cholesterol molecules³ within the bilayer, for example, impose order on the neighbouring hydrocarbon chains and at higher concentrations prevent the transition to the disordered phase. The *gauche* configurations where the chain is bent at one or several places have energy higher than the *trans*-configuration. Near the planes defined by the polar heads, the chains cannot have a random configuration for purely geometrical reasons. Finally, the degree of smectic order influences the orientational ordering¹.

I propose to describe the ordering effect of all these factors by a single parameter, η_0 , to be added to the molecular field. The one-particle potential becomes

$$V_1(\eta, \eta_0) = - V_0 \left(\frac{3}{2} \cos^2 \theta - \frac{1}{2} \right) (\eta + \eta_0)$$

In principle, η_0 is a function of the smectic order parameter which describes the tendency of polar heads to lie within the planes. However, as a first approximation, it can be assumed that the smectic order remains constant during the hydrocarbon chain transition and an η_0 independent of temperature can be

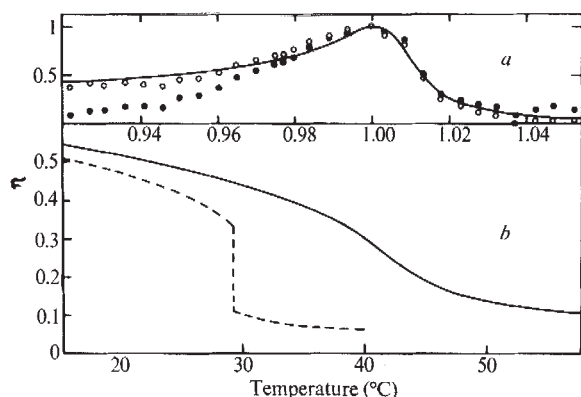


Fig. 1 (a) Specific heat of pronase-treated *M. laidlawii* membranes as a function of temperature. (—), Model result for $\eta_0 = 0.0174$; ●, data from ref. 4 with the baseline choice by the authors; ○, same data with the baseline determined by the least squares fit. (b) (—), order parameter for $\eta_0 = 0.0174$ corresponding to the specific heat data; (---), order parameter for $\eta_0 = 0.0070$.

used. This approximation is better for natural membranes where other molecules prevent large fluctuations in the position of polar heads (and thus increase the transition temperature slightly for hydrocarbon chains⁴). When the degree of smectic order is measured as a function of temperature (for example by X-ray scattering) the influence on the hydrocarbon chain order can be included in the model¹.

The order parameter η and other thermodynamic properties can now be calculated. The problem is formally equivalent to that of anisotropic molecules in an electric field discussed by Hanus⁵, where the mathematical procedure is described. The equations for η and for the entropy are

$$\eta = \frac{\int_0^1 d \cos \theta \left(\frac{3}{2} \cos^2 \theta - \frac{1}{2} \right) \exp[V_1(\eta, \eta_0)/kT]}{\int_0^1 d \cos \theta \exp[V_1(\eta, \eta_0)/kT]}$$

$$TS = -Nn V_0 \eta^2 + Nn k T \ln \int_0^1 d \cos \theta \exp[V_1(\eta, \eta_0)/kT]$$

where n is the number of independent segments for the molecule. The specific heat is given by $C = T \partial S / \partial T$.

I have not included the effect of volume changes during the transition on the thermodynamic properties. Measurements from X-ray scattering on *Mycoplasma laidlawii* membranes by Wilkins, Blaurock and Engelman⁶ show that the decrease in thickness of membrane during melting is compensated by the increase in membrane area, making the net volume change small.

Depending on the value of η_0 , the present model predicts a continuous change of order with temperature or, for smaller values of η_0 , a first-order phase transition³. The critical value of η_0 separating these two regions is $\eta_0 = 0.0103$.

Both these regimes have been observed experimentally. Natural membranes tend to show a continuous change of order, spread over larger temperature intervals if a significant fraction of cholesterol is present. Some of the artificial membranes which have more freedom for possible orientations of the hydrocarbon chain segments show clearly a first-order phase transition, such as those described by Krasne, Eisenman and Szabo⁷.

I have compared the predictions of the present model with the calorimetric data on the phase transition in membranes of *Mycoplasma laidlawii*⁴. If the maximal specific heat and the corresponding temperature are C_M and T_M , respectively, a plot of C/C_M against T/T_M depends only on η_0 , determined by a fit to the data. This is shown in Fig. 1 using specific heat data⁴ on pronase-treated membranes of *M. laidlawii*.

The agreement between the model theory and the experiment depends also on the choice of the baseline for the specific heat anomaly data, that is, the choice of the boundary between the "anomaly" and the background. I believe that the method of Melchior *et al.*⁴ is not necessarily the best as this method excludes the possibility of a term in the background which is linear in T . Such terms in the specific heat are generally present in complex systems. I have therefore shown the data with their baseline choice as well as with the baseline determined by the least-squares fit to the theory.

Using the value of $\eta_0 = 0.0174$ determined from the specific heat data, I have plotted the orientational order parameter η as a function of temperature for *M. laidlawii* membranes (Fig. 1b). The figure gives the average orientation of the hydrocarbon chain segments with respect to the preferred axis normal to the membrane plane. A solution for $\eta_0 = 0.0070$ corresponding to a first-order phase transition is also shown for comparison.

The average number of independent segments for membrane lipid molecules can be determined by comparing the experimental and the theoretical entropies. Transition entropy for the *M. laidlawii* membranes is typically⁴ about 3.6×10^3 cal mol⁻¹ corresponding to $n = 7.7$ which is a reasonable value for a typical lipid molecule with 30 or 40 CH₂ units.

The large increase of the transition entropy with critical temperature⁴ can be easily understood in the present model. As the length of the chains is increased the critical temperature rises⁸. The equivalent number of statistically independent segments is also increased in longer chains and hence the rise in transition entropy.

An independent test of the consistency of the present model is provided by the X-ray scattering data on *M. laidlawii* membranes⁶. The approximate change in the thickness of the membrane between 0°C and 40°C is 11 Å for erucate membranes and 8 Å for palmitate membranes. Assuming that $\cos \theta \sim \langle \cos^2 \theta \rangle^{1/2}$ we can calculate the change in thickness of the hydrocarbon region upon warming. I do not have separate results for the order parameter in erucate and palmitate membranes, but from Fig. 1b it follows that, throughout the phase transition, η changes from about 0.55 to 0.15. Using these values, the corresponding changes in thickness are 8.9 Å and 7.1 Å for erucate and palmitate bilayer, respectively. I believe that the small difference between the measured and the calculated values is caused by the change in polar heads configuration during the transition.

Here, I have presented a simple model which provides a quantitative description of the ordering process in biological membranes, in agreement with the existing calorimetric and X-ray scattering data. This agreement will be further improved when the influence of positional ordering of polar heads and hydrocarbon chains is included in the calculation, resulting in a more realistic picture of this transition. Measurement of order parameters as a function of temperature would therefore be valuable.

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Short Term Heritable Changes affecting Viability in *Drosophila melanogaster*

WILD type chromosomes of *Drosophila* from natural populations, when tested for viability in homozygous condition, are generally found to consist of a mixture of lethal, semi-lethal and quasi-normal chromosomes¹. Here we report an experiment carried out on semi-viable chromosomes to increase the viability by selection. We suggest that it is possible to modify the homozygote viability of some chromosomes, and that the modifications may be due to heritable changes in the chromosomes themselves, acquired over periods considerably shorter than normally expected for mutational changes.

The experiment was similar to that carried out by Dobzhansky and Spassky² in *Drosophila pseudoobscura*, except that the genetic background of *D. melanogaster* can be controlled to a greater extent, owing to the smaller number of major chromosomes and the greater availability of markers.

Second chromosomes of *D. melanogaster* were extracted from 20 wild type flies collected at five sites in the Hunter Valley district of New South Wales. These chromosomes were extracted and tested for viability in homozygous condition using the Curly/Plum method¹, modified slightly by outcrossing to ensure some variability in the genetic background³. Six of the original 20 chromosomes were chosen as having reduced viability (Table 1, column 2), and the remainder discarded.

Two replicate cultures were initiated from each of the six original lines using virgin wild type flies. These 12 cultures, each homozygous for chromosome II, were maintained in bottles for some 25 generations by tipping over large numbers of parents in each generation. These lines will be referred to as "homozygous lines".

At the same time two replicate cultures were started for each chromosome using virgin Cy/+ flies. The wild type chromosomes in these lines were maintained in heterozygous condition by using Cy/+ virgin flies as parents; all homozygous offspring were discarded. At least 20 pairs were used as parents in each generation of each line. These lines will be referred to as "heterozygous lines".

Homozygote viability was tested at two generation intervals until generation 14, and subsequently at generations 18 and 22.

Table 1 Homozygote Viabilities for Homozygous and Heterozygous Lines

Chromosome No.	Initial viability	Final viability	
		Homozygous	Heterozygous
T1	0.91	0.91 (0.89) 0.87 (0.89)	0.93 (0.88) 0.83 (0.88)
T2	0.90	0.96 (0.95) 0.94 (0.95)	0.84 (0.88) 0.91 (0.88)
T3	0.64	0.83 (0.88) 0.92 (0.88)	0.54 (0.55) 0.57 (0.55)
T4	0.37	0.89 (0.83) 0.77 (0.83)	0.00 (0.01) 0.01 (0.01)
T5	0.33	0.46 (0.53) 0.60 (0.53)	0.27 (0.20) 0.13 (0.20)
T6	0.31	0.84 (0.88) 0.91 (0.88)	0.01 (0.01) 0.01 (0.01)

Average values for replicate lines are given in parentheses.

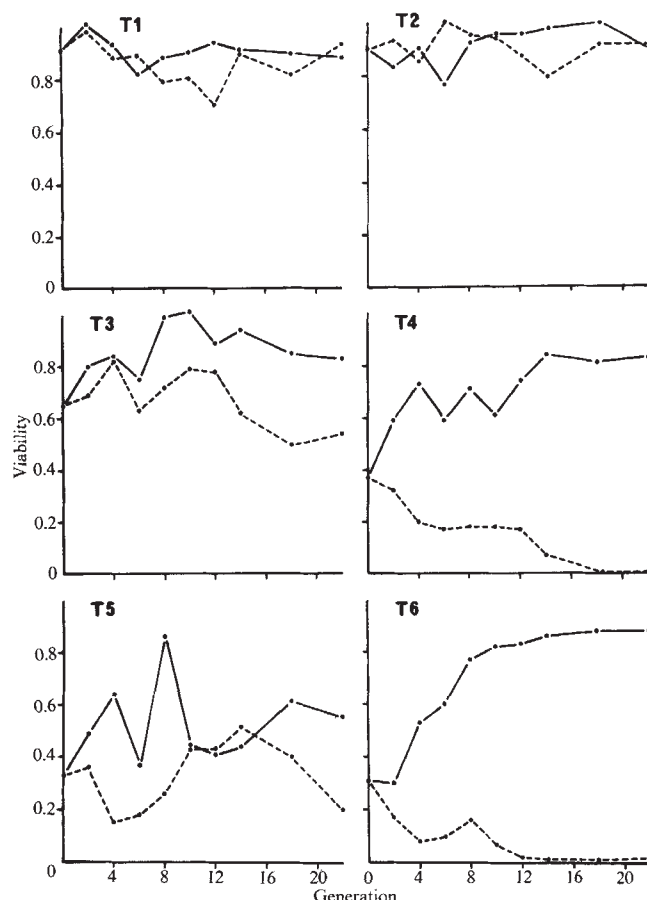


Fig. 1 Homozygote viabilities in homozygous (—) and heterozygous (---) lines from six original chromosome stocks.

In homozygous lines the Cy chromosome was introduced into each line by means of a Cy/Pm stock with genetically marked third chromosomes, including the Ubx inversion to prevent crossing over. Using the Cy/Pm stock as male parent, the Cy chromosome was introduced into the genetic background of each line, leaving only the Y chromosome and a proportion of chromosome IV uncontrolled. The equivalent test for the heterozygous lines was simpler, since Cy/+ stocks for the viability test were immediately available.

Viabilities over the course of the experiment are given separately for each chromosome in Fig. 1, in which results from replicate lines have been pooled. The viabilities attained in each line by the end of the experiment are also given in Table 1.

Chromosomes T1 and T2, those starting closest to normal viability, changed little in either homozygous or heterozygous lines. Marked changes were, however, produced in all other lines. In particular the viability rose in all cases in the homozygous lines, although considerably less in T5 than in the other lines. This rise was not unexpected, because continued use of homozygous flies as parents might have this effect under almost any selection hypothesis.

There was reduction in viability, however, in the heterozygous lines, most marked in T4 and T6 in which the homozygotes were essentially lethal by the end of the experiment. Because homozygote viability was tested and the chromosomes held as heterozygotes, the nature of the selective forces here was not clear. Competitive improvement in the heterozygote was considered but seemed unlikely, especially in view of the extreme reduction in homozygote fitness and the low conditions of competition under which the viability tests were carried out. Gametic selection was also ruled out, owing to the agreement with Mendelian expectations in all crosses between lines.

These results do not give any indication as to what extent

Table 2 Effect on Viability of Exchanging Genetic Backgrounds

	Chromosome II from	
	Homozygous lines	Heterozygous lines
Background unchanged *	0.85	0.01
Own background †	0.91	0.00
Replicate background ‡	0.84	0.02
Background exchanged §	0.84	0.01

* Viabilities within lines determined concurrently by the usual method.

† Control test of the crossing programme, chromosomes replaced in their original genetic background.

‡ Replacing genetic background with the background from replicate lines.

§ Exchanged genetic background between homozygous and heterozygous lines of the same chromosomal stock.

the changes in viability are due to changes in chromosome II, or to what extent they are due to genes on other chromosomes or cytoplasmic factors, referred to collectively as "genetic background". Conventionally, in the absence of any mutation, all of the effect must be attributable to changes in the genetic background and a crossing programme was carried out to test this. Using the genetically marked second and third chromosome stock mentioned above, it was possible in a six generation crossing programme to set up the cross $Cy/+ \times Cy/+$, with the second chromosomes coming from one line and the genetic background from another. As before, only the Y chromosome and chromosome IV were uncontrolled. Chromosomes T4 and T6 only were used for this test, as the highest differences between homozygous and heterozygous lines were found for these chromosomes.

Results are summarized in Table 2; they have been combined for all four homozygous lines and all four heterozygote lines, because these were similar. The genetic background had very little effect on the viability, which appeared to be determined essentially by the constitution of the second chromosome.

These results are difficult to explain on a conventional genetic basis. The absence of any background effect is probably not by itself of any special significance. The changes which appear to have taken place in the homozygous chromosomes, however, could only be accounted for conventionally by mutation. This mutation would have to be very specific, and to occur at a very high rate. Also, the method by which the mutations spread at such a high rate through the heterozygote lines would require some special explanation.

Some further preliminary tests were carried out, primarily on chromosomes T4 and T6. First it was found that the high viability chromosomes are in all cases dominant, or nearly so, to the low viability chromosomes. Second, that high and low viability chromosomes could be re-extracted, apparently unaffected, from male flies produced by crosses between the homozygous and heterozygous lines. This procedure was also carried out for female F1 flies, in which recombination might be expected to have some effect, but the results in preliminary tests were not very different from the male results. Interpretation of these results is further complicated by the finding that chromosomes re-extracted from the high viability homozygote lines and tested individually appeared to consist of a mixture of high and low viability chromosomes.

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Histamine and Cyclic 3',5'-AMP in Gastric Acid Secretion

IN slices from guinea-pig and rabbit cerebral cortex, histamine and a variety of compounds structurally related to histamine have been shown to enhance the formation of cyclic 3',5'-AMP (adenosine 3',5'-monophosphate)^{1,2}. The order of potency of these agents was almost identical to their order of potency in stimulating gastric acid secretion³, suggesting some similarity of receptor qualities both in cerebral cortex and in gastric mucosa, including the possibility that cyclic AMP might be an intermediate in histamine-stimulated gastric secretion.

Experiments were performed in SPF Wistar rats (180–200 g body weight). Subsequent to histamine diphosphate (HDP) application, the gastric acid output was determined using the perfused rat stomach preparation described in detail by Lai⁴. Gastric mucosal levels of cyclic AMP were measured by use of an isotope dilution procedure⁵.

Fig. 1 shows dose-response curves to HDP given subcutaneously. The gastric acid output increased with increasing doses of the stimulant. Up to doses of 6 mg of HDP/kg rat body weight a fairly typical dose-response curve was obtained compatible with previous results^{6,7}. The rise in acid secretion was paralleled by a dose-dependent increase in the cyclic AMP content of gastric mucosa. The correlation coefficient r satisfied the criteria for linear correlation between both the parameters ($r=0.972$; $P<0.01$).

To elicit temporal correlations between effects of HDP on gastric mucosal levels of cyclic AMP and on gastric acid secretion, 0.75 mg of HDP/kg rat body weight was injected rapidly into the femoral vein. Cyclic AMP contents almost doubled within 60 s, reaching near-maximal values, whereas acid secretion increased only after a lag period of 4 min (Fig. 2). This sequence of events adequately verifies one of the essential requirements set by Sutherland, Øye and Butcher⁸ to determine whether cyclic AMP is or is not involved in a particular physiological process.

There is considerable evidence that histamine interacts with the membrane-bound adenylyl cyclase system of parietal cells resulting in increased intracellular levels of cyclic AMP which as a second messenger takes over the role of histamine inside the cells. Certain xanthine derivatives known as inhibitors of the cyclic AMP degrading phosphodiesterase may potentiate the stimulatory effect of histamine on gastric acid secretion⁹,

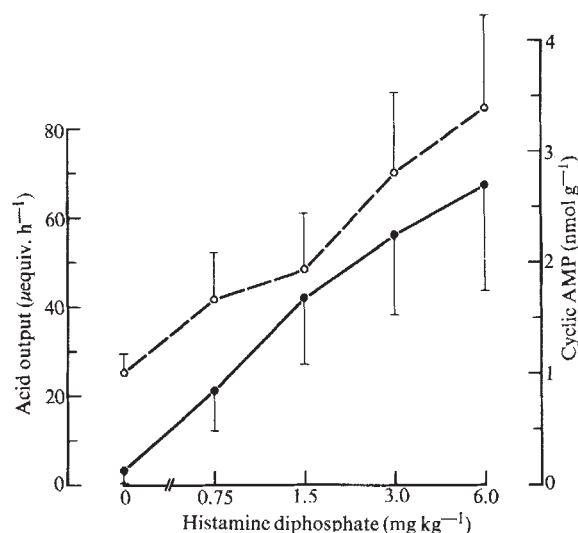


Fig. 1 Dose-response curves to histamine diphosphate given subcutaneously to rats. The scale for histamine dose is logarithmic. ○—○, Gastric acid output (μequiv. h⁻¹); ○---○, cyclic 3',5'-AMP content of gastric mucosa (nmol/g wet weight). Each point represents the mean of determinations in 6 animals ± s.d. (vertical bars).

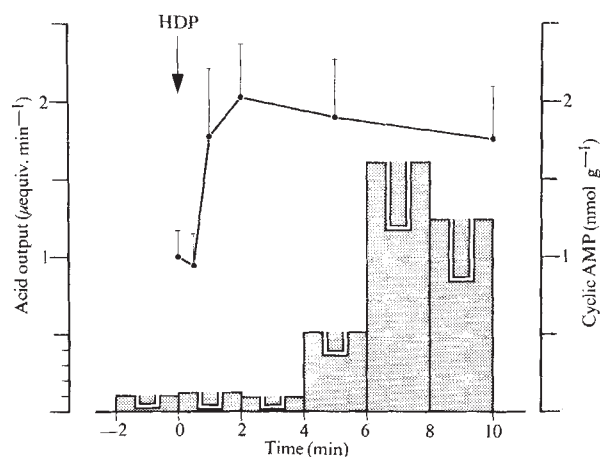


Fig. 2 Time-dependent effects of histamine diphosphate (HDP; 0.75 mg kg⁻¹) on gastric mucosal levels of cyclic 3',5'-AMP (nmol g⁻¹ wet weight) (○—○) and on gastric acid output rates (µequiv. min⁻¹; stippled blocks). Each point and block, respectively, represents the mean of determinations in 6 animals ± s.d. (vertical bars). HDP was administered to rats by rapid i.v. injection.

indicating adenyl cyclase as the most likely site of histamine attack. This was confirmed by Perrier and Laster¹⁰ and by Nakajima *et al.*¹¹ who showed increased activities of the fundic adenyl cyclase due to histamine application both in guinea-pig and in *Necturus* gastric mucosa. In dogs, however, the situation appears considerably more complex, as the adenyl cyclase from canine gastric mucosa is not activated by histamine¹².

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Parallel Correction of Cancerous Growth and of a Genetic Defect of Cell-to-Cell Communication

THREE strains of cells have recently been found which, unlike normal cells, do not make junctions of a kind that connects the cell interiors to each other^{1,2}. These three strains are also

cancerous^{1,2}. We explore here whether the junctional defect is related to the cancerous state of these cells; we fuse cells of one of the strains with normal cells that make junctions and examine the junctional permeability and growth properties of the resulting hybrids. This approach is based on the findings of Harris and Klein and colleagues, and of Weiss, Todaro and Green, that tumorigenicity is markedly reduced^{3,4} and contact inhibition of growth resumed⁵ in hybrids between various kinds of cancer and normal cells. This also turned out to be the case with the present cell systems. The question is then, is the normalization of the growth properties associated with normalization of junctional connexion? The results show that both defects are corrected in the hybrids.

Cells of four types were used: cells from a cancerous epithelial strain derived from a rat liver tumour (A strain¹), cells from a normally growing (hereafter normal) fibroblastic strain derived from the same tumour (B strain¹), a normal mutant of the B strain (see below), and cells from a normal liver epithelial line². By use of the Sendai virus⁶, the cancerous cells were fused with cells of each of the three normal types.

Junctional communication (coupling) was measured electrically and, simultaneously, by fluorescent tracer diffusion^{1,7}. The measurements were done on multinucleate hybrids, the primary products of cell fusion (before they entered mitosis); or on their descendants in which the material of the parental nuclei had condensed into a single nucleus. The primary hybrids were easily distinguished from the parental cells during the measurements by the number of their nuclei; their hybrid nature was verified, after the measurements, by autoradiography (the nuclei of one of the parental cell partners were labelled with tritiated thymidine). The measurements on the mononucleate descendants of the primary hybrids were done on pure hybrid populations: (i) clonal populations of hybrids, each derived from a separate fusion between cancer cells and liver epithelial cells; or (ii) hybrids made with a thymidine kinase-deficient mutant selected from B cell cultures in media with progressively increasing concentration (up to 50 µg ml.⁻¹) of 5-bromodeoxyuridine⁸. To obtain pure hybrid populations of kind ii, we cultured the cells in medium containing hypoxanthine, aminopterin and thymidine (HAT)⁹, in which the parental mutant cell cannot grow¹⁰ (the cancerous parental cell and the hybrid, which both have thymidine kinase, grow in this medium); we then eliminated the cancerous parental cells and the fused cancer/cancer cells by repeated passaging in Dulbecco's medium¹¹, in which the two latter types do not adhere to the dishes¹. (Aside from its usefulness for isolating a pure hybrid population, we worked with a B cell mutant here because the wild type tended rapidly to overgrow the hybrid; this was not quite so serious a problem with the liver epithelial cells.) We subsequently cloned the cells, so as to be able to measure coupling in populations the hybrid character of which had been established by karyotype. The karyotypes were examined on metaphase spreads of the clones treated with colcemid (0.2 µg ml.⁻¹, 3–5 h) after the coupling measurements. The modal number of chromosomes in the hybrids corresponded approximately to the sum of the modal numbers in the respective parental cells; moreover, the cancerous cell and the normal mutant parental cell had convenient marker chromosomes.

Tumorigenicity was tested with cell suspensions injected into the progeny of the Buffalo rats from which all parental cells had been derived. The suspensions contained normal and hybrid cells in the tests with primary hybrids, and only hybrids in the tests with later generations.

The cells were grown in plastic Petri dishes at 37° C; the cancerous parental cells in enriched F-12 medium¹² (the cells adhere to the dishes in this medium¹), the normal parental cells in Dulbecco's medium, and the hybrids in either of these two media. The two media were supplemented with 10% foetal calf serum. The coupling measurements were taken at room temperature ranging from 23°–24° C and in a few cases at 37° C with the same results.

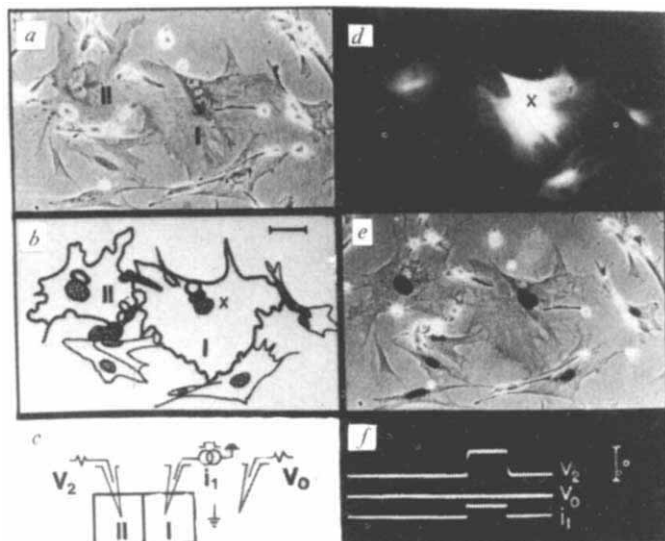


Fig. 1 Suppression of coupling defect by hybridization. Two hybrid cells (I and II) were produced by fusion of noncoupling cancer cells and coupling normal fibroblasts. The primary hybrids couple with each other and with normal fibroblasts from the parental strain. Hybrid I contains two normal fibroblast nuclei and one cancer cell nucleus. Hybrid II contains one nucleus from each parental cell. Coupling is probed electrically and with fluorescein 24 h after cell fusion. *a*, Phase contrast photomicrograph. *e*, Autoradiograph; the normal cell nuclei are ^3H -thymidine labelled. *b*, Tracing of the micrographs. *d*, Darkfield micrograph showing the results of fluorescein injection into hybrid I. Note that fluorescein has passed to hybrid II and to the adjacent normal parental cells, but not to the five parental cancer cells (the parental cancer cells are more refractile; hatched in *b*). Calibration, 50 μm . *f*, Records from an electrical measurement between two hybrids; *i*, current pulse (2×10^{-8} A, 100 ms duration) passed between interior of hybrid I and grounded exterior (*c*); V_2 , V_0 , the resulting voltage changes in hybrid II and in the exterior. Calibration 100 mV.

The parental cells were strikingly different in their growth properties. The A cell is noncoupling, its growth is not contact inhibited (it grows to densities of 10^6 cells cm^{-2} in culture) and it is highly tumorigenic (inocula of 10^2 cells produce fatal tumours in the animals). The three types of normal parental cells are coupled, contact inhibited (saturation densities $\leq 10^4$ cells/ cm^2) and nontumorigenic¹. The resulting hybrids took after their normal parents in all these respects.

Fig. 1 illustrates this for the coupling of the primary cancer cell/normal fibroblast hybrid. Two hybrid cells containing the nuclei of cancerous and normal (labelled) parents establish coupling with each other and with normal parental cells: fluorescein (330 MW) injected into one of the hybrid cells (I) is seen to have passed to a contiguous hybrid cell (II) and to four contiguous normal parental cells (there is no passage to any of the five contiguous cancerous parental cells); and a substantial fraction of the electric current injected into cell I is shown to have flowed through cell II. The junctional defect is evidently corrected in the hybrids. The example illustrated is typical of 11 cases examined. Similar results were obtained with the primary cancer cell/normal epithelial liver cell hybrids (14 cases) (Fig. 2).

The junctional defect was also corrected in the descendants of the primary hybrids. Eight clones of the cancer cell/normal (mutant) fibroblast hybrids and five clones of the cancer cell/liver epithelial cell hybrids were examined. Twenty-six coupling measurements were taken in which there was no cell membrane damage, as shown by measurement of input resistance¹ or by cellular fluorescein retention. In all these measurements the hybrids were found to be coupled to each other. (Each measurement was taken on a separate cell cluster where in each case coupling could be demonstrated by the fluorescein method for at least ten cell junctions and, in many measurements, for more than 20; Fig. 3.) The degree of electrical

coupling between the hybrid cells and the extent of coupling, as seen by the fluorescein spread, were indistinguishable from those in the normal parental cells.

The correction is not simply due to cell cultivation or to cell fusion *per se*: the cancerous parental cells remain noncoupling (and tumorigenic) after many months in culture and, at least, the primary products of cancer/cancer cell fusion are noncoupling. The correction appears to be due to the normal cell genome in the hybrid.

The correction of the junctional defect was paralleled by a correction of the growth defect. The two types of hybrids were as sensitive to contact inhibition (saturation densities $\leq 10^4$ cells/ cm^2 ; 3 clones of each hybrid type tested) as the normal parental cells and were not tumorigenic. With inocula of 10^6 cells, there was no tumour incidence with the primary hybrids (four animals injected) or their descendants (6 clones of each hybrid type tested; 12 animals injected) as against an incidence of 100% with the cancerous parental cells (36 animals). (Even inocula of 10^2 cancerous cells produced fatal tumours in four out of four animals. The incidence with the three types of normal parental cells was 0, fifteen animals.)

The results reveal a correlation between two phenotypic changes in the present cell systems: restoration of junctional coupling and restoration of normal growth. This encourages us in the idea that defective junctional coupling may be an

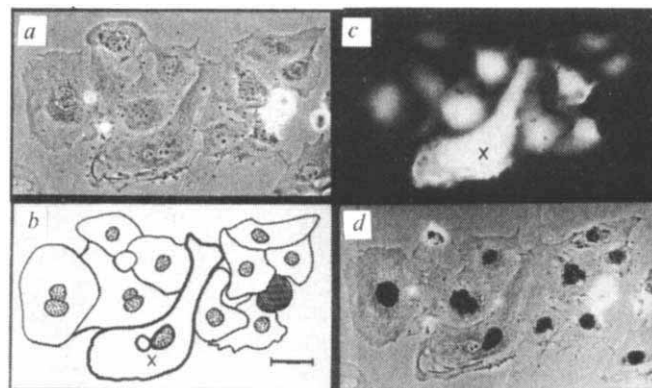


Fig. 2 A coupling primary cancer cell/normal epithelial cell hybrid. Fluorescein injection into the hybrid (X). Normal cell nuclei are ^3H -thymidine labelled (autoradiograph *d*). Parental cancer cell hatched in *b*. Calibration 50 μm .

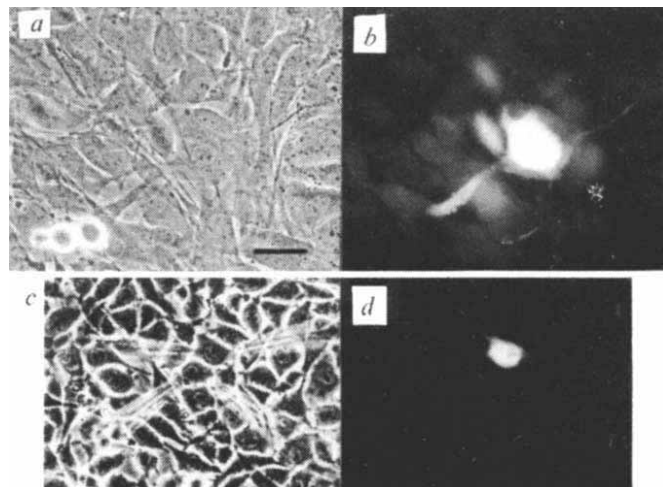


Fig. 3 Top row: coupling among cancer cell/normal (mutant) fibroblast hybrids. The cells are clonal descendants, of verified karyotypes, of the primary hybrids. The larger central cell with the long extensions was injected with fluorescein which, in *b*, is seen to have spread extensively through the clone. Bottom row: the noncoupling parental cancer cell. *b*, A fluorescein injection into a cell of a cluster of cancerous A' cells. *a* and *c*, Photomicrographs in phase contrast; *b* and *d*, in darkfield. Calibration 50 μm .

aetiological factor of certain kinds of cancer, as well as in the more general idea that the junction may be a pathway for growth-controlling molecules^{13,14}. The results do not yet establish, of course, a genetic correlation between the junctional and growth defects. To establish such a correlation, the analysis must be completed by an investigation of the segregants of the hybrids. This task, which we have just begun, will be a long one; the hybrids we have made have rather stable chromosome combinations.

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Influence of Cell-free Ascites Fluid and Adenosine 3'5'-Cyclic Monophosphate upon the Cell Kinetics of Ehrlich's Ascites Carcinoma

CELL and tissue growth regulation occurs in response to specific stimuli. Bullough^{1,2} has shown that a number of tissues contain a saline-extractable, cell-specific substance, chalone, which can depress the mitotic activity of the corresponding cells in these tissues. He concluded that the presence of adrenaline and hydrocortisone was necessary in order to activate the chalone.

Increased and abnormal mitosis is a characteristic of many malignant cells and it has been suggested that this occurs because these cells lack the inhibitory influence of chalone, due to its leakage from the cells. In four transplanted tumours studied *in vitro* it was found that the addition of the appropriate chalone extract resulted in a reduction of mitotic activity as measured by the mitotic index (MI)^{3,4}. Similar treatment *in vivo* caused regression of malignant melanoma in mice and hamsters⁵.

Bichel^{6,7} showed that cell-free fluid obtained from a mature mouse ascites tumour caused depression of MI when injected into a mouse bearing an immature tumour. He postulated that a growth regulating factor was present in ascitic fluid which appeared to produce similar effects to those of chalone.

The requirement of adrenaline and hydrocortisone to activate chalone is an interesting observation. Adrenaline is known to be involved in the production of adenosine 3'5'-cyclic monophosphate (cyclic AMP) from ATP by activation of the enzyme adenylate cyclase and cyclic AMP is believed to be involved in the action of several hormones including hydrocortisone. Evidence in the literature suggests that cyclic AMP is involved in the regulation of cell growth⁸⁻¹¹.

We have therefore performed a series of experiments on the growth kinetics of Ehrlich's ascites carcinoma (EAC) *in vitro* and *in vivo* to substantiate the concept that chalone and cyclic AMP produce a similar change in MI. We are at present attempting to characterize the chalone and determine the relationship to cyclic AMP (P. R. C., in preparation). Here we report preliminary results implicating cyclic AMP as a tumour cell growth regulator.

The EAC tumour was maintained in this laboratory by weekly intraperitoneal injection of 0.1 ml. of ascites fluid containing 1×10^8 cells. Male mice, strain C3H, were used throughout the investigation, and cells for injection were collected from mice bearing a 12 to 18 day old tumour. The cell-free chalone fluid was obtained by centrifugation of ascites fluid from a mouse with a terminal tumour, that is, one in which the tumour was greater than 21 days old. It had been found from *in vitro* experiments that the maximum response to chalone in terms of MI to ascitic chalone occurred with 14 day old tumour cells. In these experiments therefore mice bearing a 14 day old tumour were used.

The EAC cells were suspended in Hanks balanced salt solution containing 10% foetal calf serum so that the final cell count was 2 to 3×10^6 cells ml.⁻¹ of culture medium. Ascites chalone fluid (0.2 ml. or 0.5 ml.) or cyclic AMP (1, 10 or 100 μ g) was added to the appropriate tubes. In some instances adrenaline 10 μ g and hydrocortisone 10 μ g dissolved in 0.1 ml. balanced salt solution were added to the tumour cell suspension. The final volume of culture medium was 4 ml. and tumour cells were incubated in Leighton tubes at 37° C for 4 h.

Colchicine, 10 μ g, in 0.1 ml. balanced salt solution was added to each culture 3 h before harvesting to arrest the dividing cells in metaphase; 1 h was allowed to elapse between the start of incubation and the addition of colchicine, so that any cells already dividing could pass through metaphase.

Cells were harvested after 4 h incubation and processed to display mitotic figures. The cells were swollen in hypotonic medium (2 parts BSS : 8 parts distilled water) for 20 min then fixed in acetic alcohol (glacial acetic acid, 1 part : ethyl alcohol 95%, methyl alcohol 5%, 3 parts) for 20 min, resuspended in fresh fixative, spread on a microscope slide, and air dried for 30 min. The slides were then stained in 1% aceto-orcein for 30 min, dehydrated in alcohol, cleared in xylene and mounted in DPX. The mitotic index (MI) was calculated as the number of cells in mitosis per 1,000 cells. Approximately 4,500 cells were counted per sample.

The *in vivo* experiments were also carried out using mice bearing a 14 day old tumour. Chalone fluid was obtained from terminal mice as described previously.

Animals were not given adrenaline and hydrocortisone in these experiments as it was assumed these substances would be available in physiological quantities in the intact animal. Ascites chalone fluid 1.0 ml. and 0.5 ml., or cyclic AMP 50 mg kg⁻¹ and 12.5 mg kg⁻¹, was injected 4 h before sacrifice; all injections were the same volume. Colchicine 20 to 25 μ g kg⁻¹ was injected 3 h before sacrifice to arrest dividing cells in metaphase. The cyclic AMP dose levels were determined from preliminary experiments using a dose range of 25 μ g kg⁻¹ to 50 mg kg⁻¹. The cells were then processed as for the *in vitro* experiments and the MI calculated on the same basis.

Our experiments provided the following data: (1) EAC cell-free ascitic fluid obtained from a terminal tumour mouse did not inhibit EAC cell growth as measured by the mitotic index unless activated by adrenaline and hydrocortisone. The

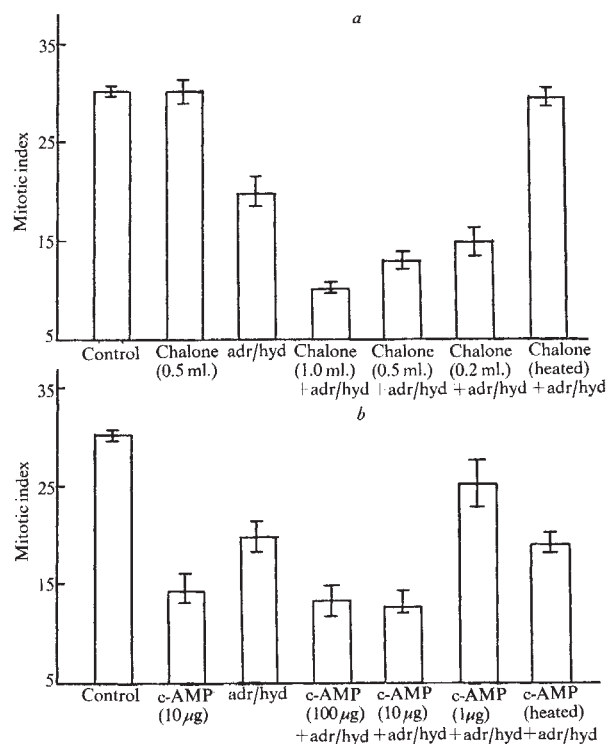


Fig. 1 Effect of (a) added chalone and (b) 3',5'-cyclic AMP on EAC cells grown in cultures.

activated fluid produced a dose-dependent depression of mitotic index (Fig. 1). Heating the chalone fluid to 100° C destroyed the inhibitory effect. (2) The addition of cyclic AMP to EAC culture produced a marked depression of mitotic index (Fig. 1). Unlike chalone fluid, cyclic AMP did not require the presence of adrenaline and hydrocortisone to produce mitotic inhibition, and the presence of adrenaline and hydrocortisone did not significantly ($P > 0.35$) increase the depression of mitotic index. A dose-dependent relationship was observed, there being a significant ($P > 0.003$) difference between the depression of MI produced by 1 µg and 10 µg of cyclic AMP. Unlike chalone fluid the mitotic inhibition produced by cyclic AMP was not significantly ($P > 0.1$) affected by heating to 100° C.

In vitro techniques can provide valid information on cell kinetics in short-term experiments before the artificial growth medium alters the tumour characteristics. The effect of biochemical change due to environment can be avoided if the tumour is studied *in vivo*.

Our experiments *in vivo* showed that both chalone fluid and cyclic AMP produced a significant depression of mitotic index over a 4 h period; chalone fluid (1.0 ml.) produced a 36.2% depression and cyclic AMP (50 mg kg⁻¹) a 47.1% depression (Fig. 2). The response in both cases was dose dependent.

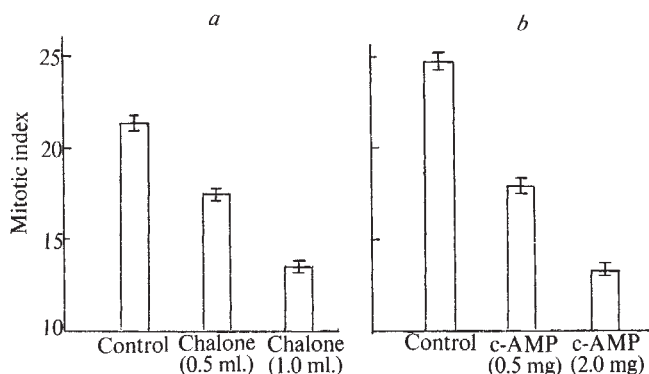


Fig. 2 Effect of (a) chalone and (b) 3',5'-cyclic AMP on EAC cells *in vivo*.

The presence of growth inhibiting factors in cell culture has been well substantiated. Growth of polyoma-transformed cells for example, is inhibited by contact with normal fibroblasts¹² and a low molecular weight constituent has been isolated which inhibited growth of transformed cells in a medium containing a low concentration of serum¹³. This substance may be related to adenylate cyclase which has been found to be deficient in transformed cells¹⁴. Thus, our own and other published data are providing evidence to suggest that the properties of chalone may be related to the adenylate cyclase/cyclic AMP system. Both regulate cell kinetics by an inhibitory reaction; both are deficient in the tumour or transformed cell; both can restore controlled growth and contact inhibition to transformed cells; both can be influenced by the same classes of drug and naturally occurring molecules¹⁵ and both chalone and adenylate cyclase are cell-specific.

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Oestrogen Receptor Binding is not restricted to Target Nuclei

WHEN oestrogen molecules enter a target cell they are bound by specific receptors in the cytoplasm which then enter the nucleus carrying the hormone¹. In the nucleus the hormone-receptor complexes bind to acceptor sites, where they presumably initiate the sequence of events comprising the hormone response.

Only target cells for oestrogen possess the specific oestrogen receptor²⁻⁴. There has been some suggestion that nuclear acceptor sites for oestrogen receptor are similarly only found in target cells⁵⁻⁸, but there are other reports that oestrogen receptor binds equally in all nuclei⁹. We have determined quantitative receptor binding to nuclei prepared from several different tissues and have examined the recovered receptor by sucrose gradient sedimentation, and we find no significant difference in acceptor activity between target and non-target nuclei.

The cytoplasmic oestrogen receptor was prepared from rat uterus and charged with ³H-oestradiol at 4° C before incubation at 25° C with crude nuclear pellets from various rat tissues (Fig. 1). Nuclear pellets from stomach, liver, or R3230AC mammary tumour retained as much receptor-bound oestradiol as nuclei from uterus, while spleen nuclei

retained only a little less. Highly purified liver nuclei showed the same binding as the crude liver nuclear pellets. The R3230AC tumour, a transplantable rat mammary carcinoma which does not depend on oestrogen for growth, has only traces of receptor¹⁰, though chromatin from this tumour is capable of extensive receptor binding^{11,12}. Assuming about 6 pg DNA per diploid nucleus in the rat¹³, all tissues tested bound at least 3,000–4,000 oestradiol molecules per nucleus.

To determine whether any qualitative differences appeared in receptor bound by target as opposed to non-target nuclei, we recovered the receptor from the nuclear pellets after cell-free incubation by extracting with 0.4 M KCl and applied the extracts to sucrose density gradients for centrifugation. Very similar sedimentation patterns were revealed in all cases (three are shown in Fig. 2). The 4–5S peak was identical to that seen for receptor from uterus nuclei after *in vivo* exposure to ³H-oestradiol¹⁴. A 3S peak which was not seen *in vivo* appeared along with the 4–5S peak after the cell-free incubation. This 3S peak was found whether crude or purified nuclei were used, but not when receptor was incubated without nuclei.

To find the origin of the 3S peak, we used the discovery

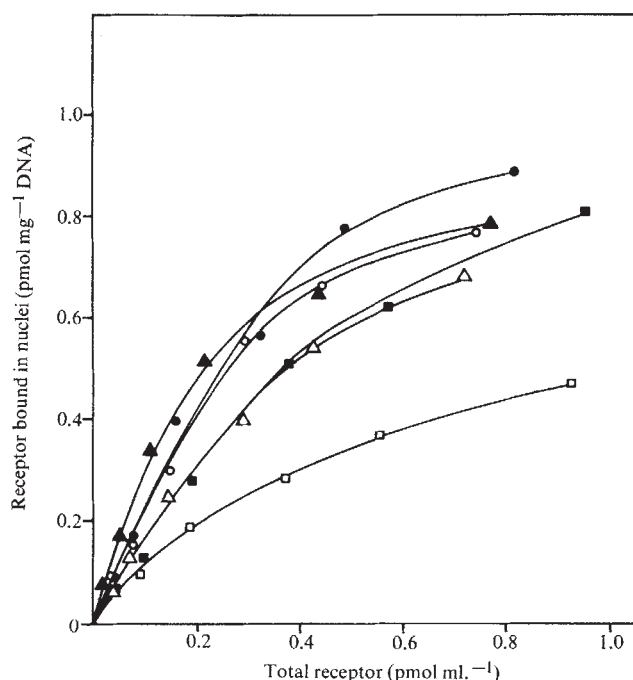


Fig. 1 Receptor bound to nuclei as a function of the total concentration of oestradiol-charged receptor (○, uterus nuclei; ●, R3230AC tumour nuclei; □, spleen nuclei; ■, stomach nuclei; △, liver nuclei; ▲, purified liver nuclei). Mature female Sprague-Dawley rats were killed 2–3 days after ovariectomy and tissues were homogenized at 4° C in 0.01 M Tris-HCl, pH 7.4, with 0.15 M KCl and 0.0015 M Na₂EDTA. Homogenates were centrifuged 40 min at 140,000g. The supernatant from uterus was charged with 1×10^{-9} M ³H-oestradiol (95 Ci mmol⁻¹, New England Nuclear) for 1 h, and remaining free oestradiol was removed with dextran-coated charcoal¹⁴. Pellets from all tissues were washed by rehomogenizing in at least 10 volumes of the same buffer and recentrifuging. Pellets containing equal amounts of DNA (50–200 pg in different experiments) were homogenized in various amounts of the charged uterine supernatant which had been diluted to 0.5 ml. with the buffer, and were then incubated at 25° C for 15 min, chilled, diluted to 4.5 ml. with the buffer, and recentrifuged. Pellets were rehomogenized and aliquots were taken for determination of bound radioactivity and DNA. Purified liver nuclei were prepared by homogenization and 96,000g centrifugation in 2.2 M sucrose with 1 mM MgCl₂ and 0.1 mM Na₂EDTA. They were incubated with charged receptor as above and trapped and washed on 'Millipore' filters which were first placed in vials for radioactivity determination and then dried for DNA determination^{21,22}.

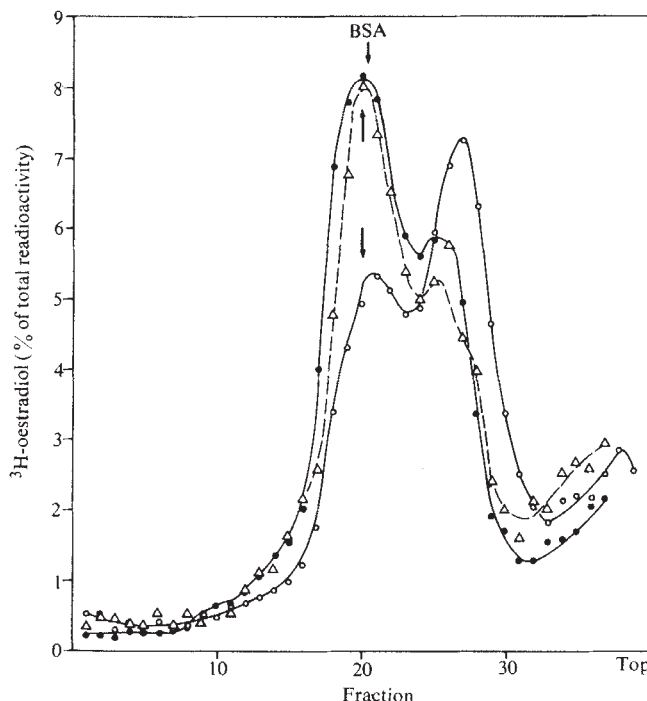


Fig. 2 Sucrose gradient sedimentation of receptor extracted from nuclei of several tissues after cell-free 25° C incubation with charged uterine receptor in Tris-EDTA, pH 7.4, 0.15 M KCl (●, uterus nuclei; ○, spleen nuclei; △, liver nuclei). Preparation and incubation followed Fig. 1 except for charging with 2×10^{-9} M ³H-oestradiol and the use of 1 ml. undiluted cytosol and about four times the previous amount of nuclear sediment. Following incubation, pellets were homogenized in 0.4 ml. Tris-EDTA, pH 8.5, 0.4 M KCl. After 60 min the extraction mixtures were centrifuged at 140,000g, and 200 µl. of each supernatant was layered on 5–20% sucrose gradients in a 'Beckman SW 56' rotor and centrifuged 15.5 h at 308,000g. ¹⁴C-Bovine serum albumin was included as a marker (arrow) in each gradient.

that receptor undergoes a temperature dependent transformation after binding oestradiol and before acting on the nucleus^{15,16}. Cytoplasmic receptor charged at 4° C can be transformed by warming to 25° C, especially in the absence of EDTA. When transformed receptor was incubated with nuclei at 4° C, the nuclei yielded a single 4–5S receptor peak like that found *in vivo* (Fig. 3). If untransformed receptor was used, however, only 3S receptor could be recovered from the nuclei (Fig. 3). A similar observation has recently been reported⁸. We conclude that the 3S binding peak arises from nuclear binding of untransformed receptor under the artificial conditions of cell-free incubation.

We therefore examined nuclei from uterus, spleen, liver, stomach and R3230AC tumour after incubation at 4° C with transformed receptor. Similarly no difference in sedimentation between receptor from target and non-target nuclei appeared (Fig. 4). Binding curves obtained with this procedure were very similar to those already seen in Fig. 1.

These experiments reveal no differences between target cells and non-target cells, either in the ability of their nuclei to bind oestrogen receptor, or in the characteristics of the receptor after being released from binding. Published results for progesterone receptor binding to chromatin^{7,17,18} and androgen receptor binding to whole nuclei¹⁹ or chromatin²⁰ have suggested that more receptor is bound by target than non-target nuclear components in those systems, but significant binding to non-target nuclei or chromatin is also seen. Even though we now question the evidence that binding sites for oestrogen receptor are restricted to target tissue nuclei, the basic concept of steroid hormone initial action is essentially unchanged and the cytoplasmic receptor remains the primary determinant of tissue responsiveness. One might ask whether non-target nuclei

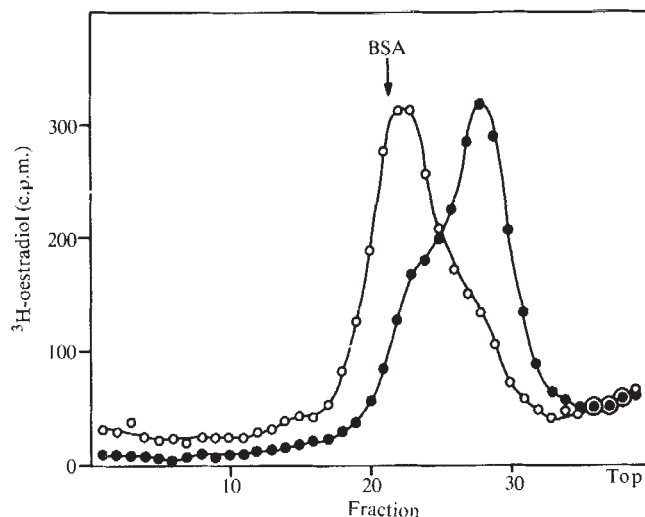


Fig. 3 Receptor extracted from uterus nuclei after incubation of nuclei at 4° C with transformed (○) or untransformed (●) cytoplasmic receptor in Tris-HCl, pH 7.4. Procedure followed Fig. 2 except for preparation and incubation in Tris-HCl, pH 7.4, incubation at 4° C for 60 min, and in one case warming of supernatant containing cytoplasmic receptor to 25° C for 30 min after charging at 4° C for 30 min.

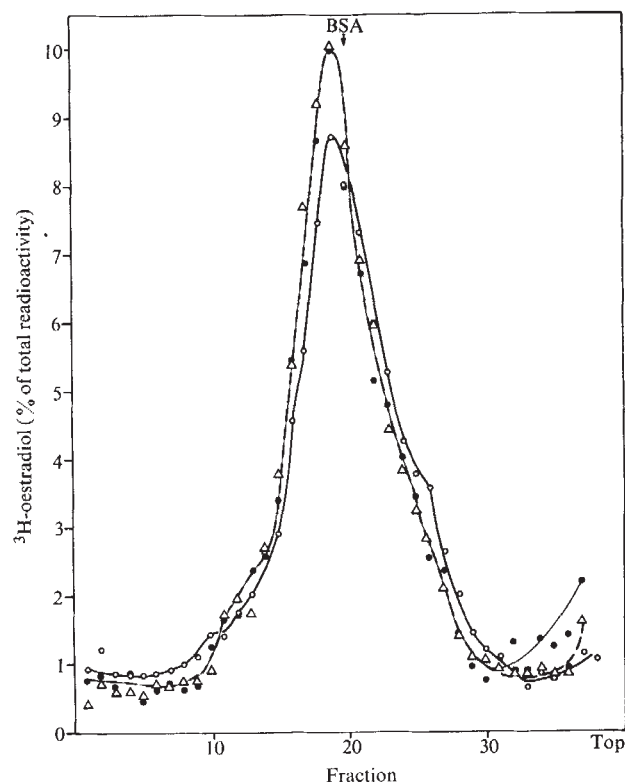


Fig. 4 Receptor extracted from nuclei of several tissues after incubation of nuclei at 4° C with transformed cytoplasmic receptor in Tris-HCl, pH 7.4 (●, uterus nuclei; ○, spleen nuclei; △, liver nuclei). Procedure followed Fig. 3, including warming of the cytoplasmic receptor.

could exhibit at least some of the characteristic responses to hormone if the receptor were provided. The experiments of Mohla *et al.*¹⁶ show stimulation of RNA polymerase activity by charged oestrogen receptor in uterus nuclei but not liver or kidney nuclei, though polymerase activity is already much higher in the latter tissues than in unstimulated uterus. No other evidence is available at present.

High affinity binding of a receptor in the nucleus has not yet been proved to be directly involved in mediating the

hormone response, nor has the specificity of the nuclear binding sites been rigorously demonstrated. It may be possible that other activities of the receptor in the nucleus or even in the cytoplasm, undetected by present techniques, have major roles in determining the response.

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Retardation of Foetal Growth and Plasma Protein Development in Foetuses from Mice injected with Coxsackie B3 Virus

RETARDED intrauterine growth and low birth weight have been observed in association with congenital defects and foetal infection in humans¹⁻⁴ and, although animal model systems are available for the study of non-infective factors related to intrauterine growth retardation⁵⁻⁷, the effect of pathogens on this aspect of impaired foetal development has not been the subject of much experimental investigation. Respiratory virus infection induced by parainfluenza 1 virus in rats causes foetal growth retardation owing to the maternal illness without

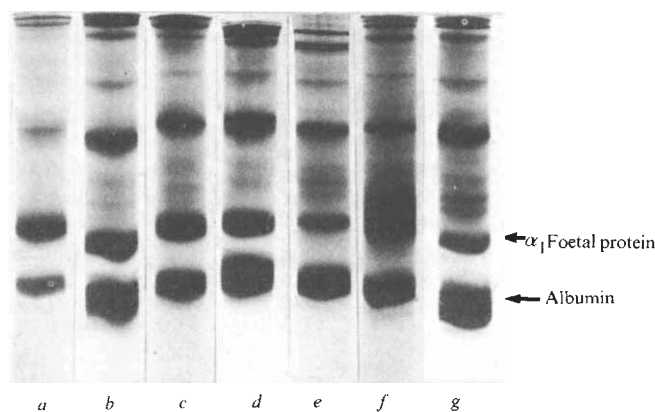


Fig. 1 Electrophoretic patterns of foetal and neonatal mouse sera. Disc gels of: *a*, 15 day foetus, control: non-infected; *b*, 18 day foetus, control: non-infected; *c*, 18 day foetus, infected: live virus; *d*, 18 day foetus, control: inactivated virus; *e*, 2 day neonate, control: non-infected; *f*, 2 day neonate, infected: live virus; *g*, 2 day neonate, control: inactivated virus. Electrophoretic conditions were: gels, 8% acrylamide containing 5% bisacrylamide; buffer, 0.037 M Tris/HCl of pH 8.6.

virus infection of the foetus⁸. The work described here, however, was carried out to study the effect of maternal infection on foetal growth and development with a virus previously shown to cause foetal infection in mice⁹⁻¹¹ without causing overt maternal illness.

Mice, eight days pregnant, were injected subcutaneously or i.p. with a dose of Coxsackie B3 virus between 10^5 and 10^6 tissue culture infective doses (TCID₅₀). Control animals were not treated or were given the same suspension of virus, inactivated by heating at 56° C for 30 min. The average weight of 142 foetuses, removed at 18 days gestation, from twenty-seven virus-treated mice was $0.952 \text{ g} \pm 0.057 \text{ g}$, compared with an average weight of 101 foetuses from fourteen control mice of $1.214 \text{ g} \pm 0.078 \text{ g}$. The difference between the two groups is highly significant ($P < 0.01$) and the maternal infection results in a reduction of foetal weights. During the experiments the pregnant mice remained in good health and did not appear to suffer any effects from the virus inoculations.

Serum levels of α -foetoprotein may be used as an indication of gestational age in human infants¹². In the present experiments disc electrophoretic analysis of the plasma proteins revealed a low ratio of albumin/ α -foetoprotein concentration in twenty-four out of thirty-four foetuses removed at 18 days gestation, compared with the ratio of these components in the plasma of foetuses from control mice (Fig. 1). It is also of interest to note that the ratio of albumin/ α -foetoprotein concentration of the two day neonate from an infected animal is similar to that of a 15 day foetus from a non-infected animal, thus indicating that the maternal infection may have interfered with the development of the plasma proteins.

We therefore suggest that inapparent maternal infection during pregnancy may be one of the causes of retarded intra-uterine growth and that a low ratio of albumin/ α -foetoprotein concentration may be an indication of neonatal immaturity in animals and human infants.

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Teratology Studies on Methylmercury Hydroxide and Nitrilotriacetate Sodium in Rats

PRELIMINARY United States government studies in 1970 indicated that trisodium nitrilotriacetate (NTA) might enhance matricidal, foeticidal, and teratogenic effects of cadmium and mercury in rats. As a result, major detergent manufacturers voluntarily discontinued the use of NTA as a detergent builder pending further investigations. Several studies have been made to reinvestigate whether NTA enhances the toxicity of heavy metals. Oral administration of NTA with cadmium chloride actually reduced the maternal toxicity of the cadmium, inhibited tissue deposition of cadmium, and had no effect on the extent or type of teratogenicity of the metal¹. The effect of NTA on maternal and foetal toxicity and teratogenicity of methyl mercury hydroxide has now been determined.

Alkyl mercurial compounds have induced a wide variety of embryo-toxic manifestations in laboratory animals. Subcutaneous administration of 40 mg ethyl mercuric phosphate kg⁻¹ body weight to pregnant mice on the tenth day of gestation resulted in gross teratogenic effects². Histological changes in the cerebral cortex of offspring of rats treated orally with about 0.8 mg kg⁻¹ methyl mercuric chloride have been reported³, and large oral doses of up to 20 mg kg⁻¹ Hg resulted in offspring with distorted cerebellar lobes⁴.

In our study, mating, administration and teratological procedures were carried out exactly as previously described¹.

Maternal mortality was dose dependent in both treatment groups. At doses 1.5–6.0 mg Hg kg⁻¹ fewer animals died in the groups given NTA than in those given sodium sulphate. All the females given 8.0 mg Hg kg⁻¹ in combination with either NTA or sodium sulphate died. Gross autopsy of animals dying during the experiment revealed classical signs of mercury intoxication although no differences in symptomology were observed between treatment groups.

In both treatments, the number of viable foetuses per female at sacrifice decreased with increasing levels of mercury. The apparent reduction in number of live foetuses for females treated with 4.5 mg Hg kg⁻¹ plus NTA as compared to the same mercury level plus sodium sulphate could be accounted for by significantly lower numbers of corpora lutea and, therefore, fewer implantation sites in these females. Ovulation and implantation occurred before dosing and could not have been affected by experimental treatment. The higher levels of mercury in each treatment tended to reduce foetal weight. Foetal weight data, however, are confused by the different incidences of foetal oedema among the test groups, and it is therefore difficult

Table 1 Effects of Orally Administered Methylmercury Hydroxide plus Sodium Sulphate or NTA from Days 9–19 of Gestation on Uterine and Foetal Parameters

Dose levels mg kg ⁻¹ day ⁻¹			Rats (No.)		Autopsy findings mean/female			Living young at day 20			
CH ₃ HgOH*	Na ₂ SO ₄ †	NTA	Pregnant females on gestation ‡ day 6	Succumbed§	Corpora lutea	Implantation sites	Resorption sites	Total No. foetuses	Viable foetuses per litter	Mean weight of young (g)	Externally malformed (%)
1.5	7.2	—	14	0	11.2	10.1	0.7	135	9.6	3.90	0
3.0	14.5	—	16	0	11.9	10.6	1.0	154	9.6	3.25	2.6
4.5	21.7	—	16	6	12.7	12.3	1.5	108	10.8	3.35	100.0
6.0	29.0	—	14	8	10.3	7.7	0.3	1	0.3	3.30	100.0
8.0	38.7	—	21	21	—	—	—	—	—	—	—
1.5	—	9.4	17	0	11.9	10.7	0.6	171	10.1	3.70	0.6
3.0	—	18.9	18	0	11.1	10.6	0.7	179	9.9	3.30	0
4.5	—	28.4	16	3	9.7	8.6	3.3	69	6.3	2.95	30.4
6.0	—	37.8	18	0	12.1	11.0	8.1	52	2.9	3.05	21.2
8.0	—	50.3	21	21	—	—	—	—	—	—	—
Controls	—	—	12	0	12.7	11.1	0.8	125	10.4	3.80	0

* Levels expressed as level of mercury.

† Added at a level to provide sodium concentration equivalent to that contributed by the trisodium NTA.

‡ Charles River COBS rats.

§ The sodium sulphate group is different from the NTA group ($P=0.01$) using a pooled 2×2 chi square test⁵.

|| Included gross oedema, haematoma of abdominal surface, haematoma of ocular area, runts, and lack of tail.

to make meaningful comparisons of the two treatments on the basis of foetal weights.

The incidences of external foetal abnormalities increased with increasing dose levels of mercury when combined with sodium sulphate. All foetuses from mothers given 4.5 or 6.0 mg Hg kg⁻¹ with sodium sulphate were abnormal, but in the NTA group, only 30 and 21% of the foetuses respectively exhibited external abnormalities.

Skeletal and internal teratology examinations are shown in Table 2. The primary skeletal abnormalities were rib defects, the incidence of which did not differ substantially between sodium sulphate and NTA combination. Foetal skeletal calcification was delayed in a dose related manner by prenatal exposure to mercury. Sternum sections that were not completely stained with calcium positive Alizarin dye were classified as incompletely ossified or non-ossified sections. The delays in calcification were similar at equivalent mercury levels in both treatment groups.

The major internal abnormalities involved defects of the brain, heart and testes. The incidence of brain defects was dose related although no significant differences were

noted between test groups at any levels of the mercury tested. Heart abnormalities were dose related in the mercury/sodium sulphate group but not in the mercury/NTA group. The frequencies of testicular abnormalities were similar for the two treatment groups at each level of mercury tested. The lack of sufficient numbers of foetuses from dams dosed with the 6.0 mg Hg kg⁻¹ plus sodium sulphate precludes meaningful comparison between the two groups at this mercury level. Other internal abnormalities observed included reduction in urinary bladder size, microphthalmia, kidney ectopia and cleft palate. The incidence of these abnormalities, however, was too low to permit their use as criteria for comparison of NTA and sodium sulphate groups.

Our observations show that orally administered methylmercury hydroxide from days 6 to 19 of gestation was toxic to the mother and foetus and was teratogenic in rats. Co-administration of NTA with methylmercury hydroxide provided protection against the maternal toxicity of mercury ($P=0.01$) and had no effect on the foetal toxicity or the type and extent of the mercurial teratogenicity. We con-

Table 2 Incidences of Foetal Abnormalities in Rats Receiving Oral Doses of Methylmercury Hydroxide plus Sodium Sulphate or NTA on Days 6–19 of Gestation

Dose levels mg kg ⁻¹ day ⁻¹			Foetal skeletal development		Foetal internal development			
CH ₃ HgOH*	Na ₂ SO ₄	NTA	No. foetuses	Rib %† abnormalities	No. foetuses	Brain %‡ abnormalities	Heart %§ abnormalities	Testicular % abnormalities
1.5	7.2	—	79	2.5	56	0	0	0
3.0	14.5	—	102	5.9	52	7.7	13.4	19.2
4.5	21.7	—	73	28.8	35	8.6	43.8	40.0
6.0	29.0	—	1	0	1	100.0	100.0	0
1.5	—	9.4	110	1.8	61	1.6	26.2	1.6
3.0	—	18.9	75	4.0	104	2.9	8.6	13.5
4.5	—	28.4	46	23.8	23	17.4	13.0	47.8
6.0	—	37.8	32	25.0	20	25.0	15.0	20.0
Controls	—	—	85	2.4	40	0	12.5	0

* Levels expressed as level of mercury.

† Included supernumerary, angulated and shortened ribs.

‡ Included hydrocephalus, blood clot in brain and compression of brain.

§ Included small, large and missing atria, compressed and small ventricles, small, large and transposed heart.

|| Included undescended, partially descended, and small testes. Approximately one-half of the foetuses from each group were males→.

clude, therefore, that ingested NTA does not increase toxicity or teratogenicity of ingested methylmercury hydroxide in rats.

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Hexosaminidase-A and Hexosaminidase-B: Studies in Tay-Sachs' and Sandhoff's Disease

HUMAN tissues contain two isozymes of β -D-N-acetyl-hexosaminidase¹. Hexosaminidase-A (hex-A) is thermolabile and moves toward the anode on electrophoresis at pH 6.0, and hexosaminidase-B (hex-B) is relatively thermostable and moves toward the cathode. In Tay-Sachs' disease, which is found predominantly in Jewish populations, hex-A is absent but hex-B activity is increased²⁻⁵. In Sandhoff's disease, a variant of Tay-Sachs' disease, found mostly in non-Jewish populations, both enzyme activities are missing^{3,6}. Thus, while it is apparent that there is a genetic relationship between hex-A and hex-B, the nature of this relationship has remained obscure. It has been suggested that hex-A may be derived from hex-B by the addition of neuraminic acid residues^{1,7}.

We have now purified human placental hex-B to homogeneity and hex-A to a very high state of purity. Antibody production against hex-A and hex-B has made possible further knowledge of the relationship between these two enzymes and the diseases which occur in their absence. Our studies of the purified enzymes and the antibodies produced against them resulted in the following three findings, which must be taken into account in any genetic and biochemical model explaining the relationship between these disorders.

First, although incubating purified hex-A with *Clostridium perfringens* neuraminidase at 37° resulted in the formation of a new, thermostable band resembling hex-B in electrophoretic mobility, the same band was also formed at the same rate in the absence of neuraminidase. Either in the absence or presence of neuraminidase only 5 or 6% of hex-A was converted to the more cathodic enzyme.

Second, as previously reported^{8,9}, antiserum produced against hex-B reacted with both hex-B and hex-A. Similarly, antibody produced against hex-A reacted with both hex-B and hex-A. We have made a specific anti-hex-A antiserum by treating serum produced against hex-A with purified hex-B. The converse, however, is not true. Treatment of anti-hex-B serum with hex-A resulted in absorption of all antibody activity.

Third, immunoelectrophoresis against anti-hex-B (which reacts both against hex-A and hex-B) gave two distinct arcs with normal liver and with liver from a patient with Sandhoff's disease. These arcs corresponded to the position of hex-A and hex-B activity. Only one arc corresponding to hex-B was found with liver from two patients with Tay-Sachs' disease. After

Table 1 Immunoelectrophoresis of Hexosaminidase A and B from Normal Tay-Sachs' and Sandhoff's Disease Liver Samples

Liver sample	Before treatment with antiserum		After treatment with antiserum and elution			
	Enzyme activity corresponding to mobility of:		Arc corresponding to mobility of:		Arc stained for enzyme activity corresponding to mobility of:	
	Hex-A	Hex-B	Hex-A	Hex-B	Hex-A	Hex-B
Normal	+	+	+	+	+	+
Tay-Sachs'	0	+	0	+	0	+
Sandhoff's	0	0	+	+	0	0

elution of non-precipitated proteins from the immunoelectrophoresis slides, the arcs formed with normal liver or the purified enzymes stain for hexosaminidase activity using 4-methylumbelliferone- β -D-N-acetyl-glucosamine substrate. In Tay-Sachs' liver, the single hex-B arc also stained for enzyme activity, but the arcs formed against liver from Sandhoff's disease were, of course, devoid of enzymatic activity.

Our findings are compatible with several models, but it seems unlikely that hex-B is merely the aneuraminyl derivative of hex-A. It seems more probable that hex-A and hex-B share a common subunit. Sandhoff's disease would result from a mutation of the active site of the common subunit and Tay-Sachs' disease from a mutation affecting a subunit present only in hex-A. Thus, hex-A may contain two different subunits ($\alpha\beta$)_n while hex-B has only one of these subunits ($\beta\beta$)_n. This would explain the cross reactivity of antiserum produced against these two forms of enzyme, and the fact that a specific anti-A serum but not a specific anti-B serum can be produced. It also explains the increased amount of hex-B which is found in Tay-Sachs' disease, since uncombined β subunits would be available.

Details of these studies will be published elsewhere.

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Another View of Neutral Alleles in Natural Populations

JOHNSON¹ has suggested that the relationship observed between the actual and effective numbers of alleles in certain natural populations is not consistent with the selectively neutral allele model of Kimura and Crow². We show here, however, that the data from a large number of natural populations are in fact compatible with this model.

If N is the effective size of a population, u the neutral mutation rate at a locus, n_a the actual number of alleles at the locus

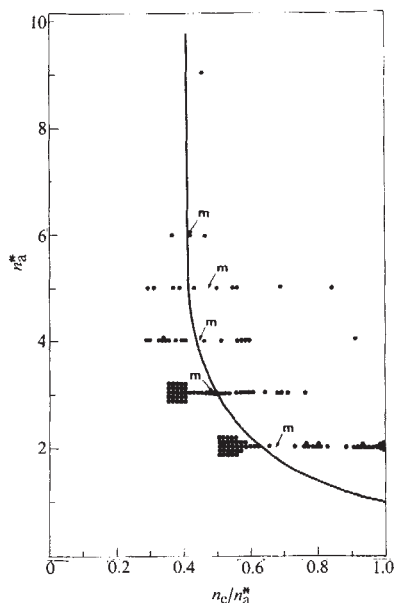


Fig. 1 Theoretical curve for n_a^* versus n_e/n_a^* , and calculated points from natural populations. n_a^* , Number of alleles with frequency ≥ 0.01 ; n_e , $1/(\text{sum of squares of allele frequencies})$; m , mean value of n_e/n_a^* for each value of n_a^* . A point on the graph generally represents data from one locus in one species. *Drosophila willistoni*³—22 loci; *D. simulans*⁴—10 loci (*D. melanogaster* rejected because two populations were not comparable, *D. affinis* and *D. athabasca* samples too small); *D. paulistorum*⁵—4 semispecies treated separately—up to 10 loci per semispecies, 29 loci in all; *D. obscura*⁶—14 loci; *D. ananasae*⁷—4 loci; *D. pseudoobscura*⁸—11 loci; *Peromyscus polionotus*⁹—5 loci; *Mus musculus musculus*¹⁰ (Denmark)—6 loci; *Mus musculus brevisrostris*¹¹ (California)—11 loci; *Mus musculus*¹² (Skokholm Island)—*Pep-C* locus only; *Astyanax mexicanus*¹³—9 loci; *Phasianus colchicus*¹⁴—*Tf* locus; *Limulus polyphemus*¹⁵—9 loci; *Clethrionomys gapperi athabasca*¹⁶—*Tf* and *Al* loci.

and n_e the effective number of alleles, then the relationship between n_e and n_a used by Johnson¹ applies when polymorphisms with the same value of $4Nu$ are studied. If, however, many loci in a population are considered, it is highly unlikely that the mutation rates will be the same at all loci, if only because of variation in the numbers of nucleotides per cistron. Also, populations will differ in effective population size. Thus $4Nu$ will vary when data from different loci in one or more populations are used, and this must be taken into account when actual data are compared with the predictions of the model.

When a sample is drawn from a population the actual number of different alleles in the sample will be less than that in the population, because rare alleles will tend to be missed. Because the effective number of alleles is little modified by the presence or absence of rare alleles, the ratio of the effective number to the actual number of alleles observed in a sample will be greater than that in the population. This bias is increased if only alleles with frequencies of 0.01 or greater are counted.

If, however, we consider only the actual number of alleles with frequencies of 0.01 or greater (n_a^*), then from Kimura and Crow², the appropriate formula for the expected value of n_a^* is

$$n_a^* = 4Nu \int_{0.01}^{1.0} (1-x)^{4Nu-1} x^{-1} dx$$

From this equation, and the relation² $n_e = 1 + 4Nu$, the values of n_a^* and n_e can be calculated for a range of values of $4Nu$. This gives the relationship between n_a^* and n_e/n_a^* shown by the curve in Fig. 1. Data from natural populations can then be compared with this relation.

Data for this comparison were obtained from a number of sources³⁻¹⁶ subject to the following restrictions: (a) allele

frequencies at each locus summed to within 1% of 1.0; (b) more than 100 genes were sampled per locus (actual samples ranged from 112 to 7,696 genes); (c) data from laboratory colonies more than six generations removed from the wild were rejected.

It is also necessary to place some restriction on the sampling unit used. Sets of data on a particular locus from two different (but not reproductively isolated) populations of a species cannot be regarded as independent, since there will generally be a high correlation between the gene frequencies in the two populations. We have adopted the procedure of pooling allele frequencies from all available samples in a clearly defined species, after weighting by sample size. Where there is evidence of a high level of reproductive isolation between two or more populations of the same species, however, these have been treated separately; for example, *Mus musculus musculus* from Denmark and *M. m. brevisrostris* from California, and the four semispecies of *Drosophila paulistorum*.

The results from over 130 polymorphisms are presented in Fig. 1. The mean value of n_e/n_a^* is indicated for each value of n_a^* . A random sample of fifty-seven is presented from the large number of diallelic polymorphisms available.

There is a close correspondence between the observed means and the theoretical curve, suggesting that the data are not inconsistent with the neutral allele model. We do not attach much significance to the wide range of values of n_e/n_a^* since computer simulation of neutral alleles (G. C. K., unpublished data) gives similar results.

A model for mutually heterotic alleles in a finite population has also been put forward by Kimura and Crow². Preliminary analysis of this simple model shows that although the relationship between n_e and n_a^* may be more complex than in the neutral allele model, nevertheless in some cases at least, the relationship between n_e and n_a^* may not differ greatly from that predicted for neutral alleles.

Pending further studies of other models, we therefore suggest that the relationship between n_e and n_a^* presented in Fig. 1 should not be used in attempts to discriminate between neutral polymorphisms and those undergoing selection.

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Isolation and Identification of the Causative Agent of "Red Disease" of Pike (*Esox lucius* L. 1766)

RED disease has been described as an acute disease of pike fry, with unknown aetiology, causing severe losses in Dutch pike culture¹. Its name was derived from the haemorrhagic lesions in the trunk, usually situated above the pelvic fins, and visible as bilateral red swollen areas in the diseased fry. In a serious outbreak during the spring of 1972 at a hatchery near Lelystad, only 0.6% of about 1,850,000 cultured young pike reached a length of 4 to 5 cm and could be used for stocking inland fishing waters. About 86% of the total mortality rate was due to red disease. Healthy fry placed in the same tank as diseased fry became infected, and it was concluded that the disease could be transmitted through water. No evidence was obtained that the disease was caused by bacteria. Here we describe work which demonstrates that the disease is caused by a rhabdovirus.

Diseased living fry showing characteristic haemorrhagic lesions were removed from the hatchery tanks, cooled in ice water, and transported to the laboratory. The fish were then packed in aluminum foil, frozen in liquid nitrogen, and stored in a refrigerator at -80°C . About two months later the frozen

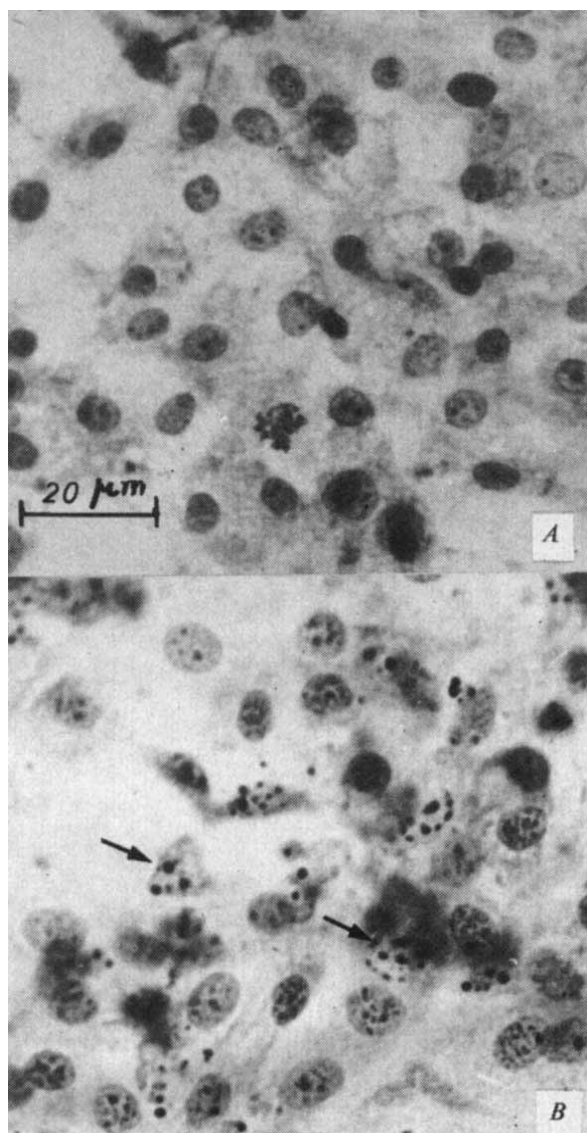


Fig. 1 CPE on FHM cells. *A*, Uninfected cells; *B*, cell changes 2 days post infection by 1 : 500 diluted filtrate from naturally diseased fries. Arrows indicate the granular degeneration of infected cells which precedes the destruction of the cell sheet.

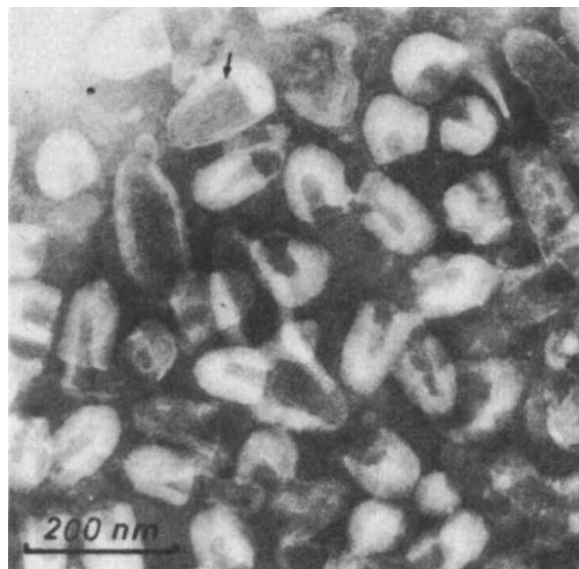


Fig. 2 Bullet-shaped viral particles from the second passage on FHM. The striations are distinguishable (arrow).

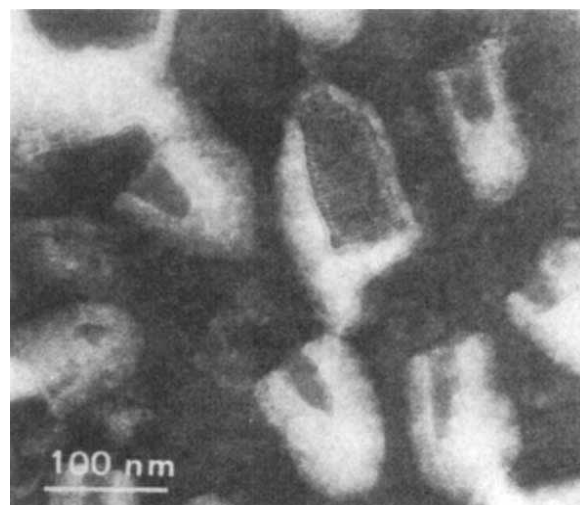


Fig. 3 Higher magnification view of one of the virus particles.

material was again placed into liquid nitrogen, and transported to France.

Ten frozen fishes were thawed in Earle's solution, with Tris buffer at pH 7.6. After complete thawing, the liquid was removed and samples were ground in a mortar and diluted 1 : 50 in cold Earle's solution. The suspension was centrifuged at 4,000*g* for 15 min and the supernatant filtered through a 450 nm 'Millipore' filter. The filtrate was used for infecting healthy pike fry and fat-head minnow cells². The cells, grown in Leighton tubes, were infected with virus and, after 30 min adsorption, washed and 1.5 ml. of Eagle's medium (without serum but supplemented with the antibiotics penicillin, streptomycin and kanamycin) then added. The tubes were incubated at $+14^{\circ}\text{C}$ and checked daily for cytopathic effect (CPE) by comparison with control cells.

Strong CPE, involving cell rounding and monolayer destruction, resulted within 40 h (Fig. 1). Infection with ten-fold dilutions allowed us to observe the evolution of the CPE which was similar to that described for spring viraemia of carp (SVC) virus³.

Electron microscopy of phosphotungstic acid stained pellets from the second passage in FHM cells established the presence of numerous bullet-shaped virus particles (Fig. 2) with one hemispherical and one planar end and an axial channel, partially penetrated by the stain. An envelope with projections

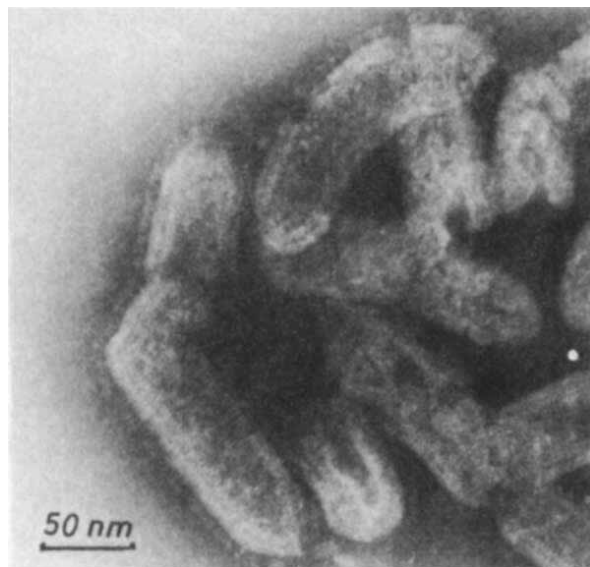


Fig. 4 Transverse striations and external projections observed on long forms of viral particles.

surrounded transverse striations running along the cylinder (Figs. 3 and 4). Dimensions were: length 125 ± 10 nm, overall diameter 80 ± 8 nm, channel diameter 25 ± 5 nm, and projection length 9 ± 2 nm.

Evidence that the disease was caused by an infectious agent was provided by intraperitoneal inoculation of four groups of young pike with (1) the initial isolate, (2) and (3) the supernatant from the second passage in FHM cells, (4) SVC virus. Control groups were inoculated with Eagle's medium. The different groups were checked for mortality, external lesions, virus isolation and histopathological changes. Moribund fishes were fixed in Bouin's solution for three days, embedded in paraffin and stained with haematoxylin-eosin. The results of this experiment are summarized in Table 1 and the mortality curves are given in Fig. 5. Only the infected fishes died, while those of the control groups remained healthy. Virus was recovered only from diseased pike.

Histological examination from groups 1 and 4 revealed lesions identical with those described in hydrocephalus and red disease of pike fry¹, tubular degeneration and necrosis of the kidney and haemorrhages in the connective tissue of the muscles. These lesions were less acute in the group infected with SVC virus.

The samples from the Netherlands used for the first isolation and transmission experiment had a titre of virus averaging 10^7 plaque forming units (p.f.u.) per 0.1 g of body weight of fish. The virus was re-isolated only from the experimentally infected fishes of the first and second groups; the other two were untested but most of the fries exhibited the macroscopic haemorrhages found in the natural disease. The experimental and natural infections produced the same histological lesions. The variations observed in the mortality rates could be due to the viral input; 10^6 p.f.u. per fish were inoculated in the first

experiment and 1.2×10^7 in the second and third. In the latter case, the work was carried out at a water temperature ranging between $+21.5^\circ \text{C}$ and $+24^\circ \text{C}$, which could explain the differences observed in the course of the disease.

It is unlikely that intraperitoneal inoculation is the natural route of infection. Because pikes spawn in late winter, and we received the samples in June, we were not able to find small pike fry (2 cm long) to demonstrate transmission by the water route. The natural disease mainly attacks young fry and we were running the risk of failing in experimental transmission with larger fry as we do not yet know anything about the usual age of infection.

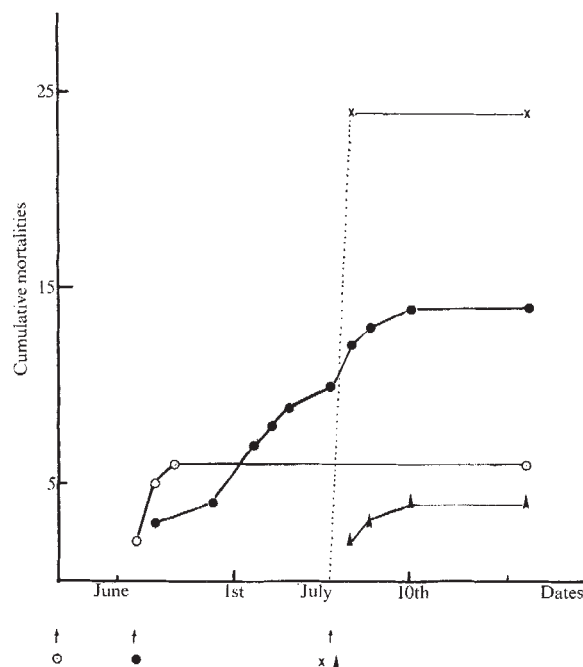


Fig. 5 Infection mortality curves. Arrows indicate the dates of inoculation. ○, First experimental group total fifteen fish; ●, second experimental group total fifteen fish; ×, third experimental group total twenty-five fish; ▲, fourth experimental group total seven fish.

In view of the knowledge of other fish diseases induced by rhabdoviruses, such as viral haemorrhagic septicaemia⁴⁻⁶ (VHS), infectious haematopoietic necrosis⁷ (IHN) of Salmonids or SVC⁴, it seems likely that the pike virus is carried by adult fishes, spread into the water and, during the spawning time, onto the surface of the eggs, involving propagation of the disease very early in hatching time.

Morphologically, pike virus undoubtedly belongs to the rhabdovirus group, but it is still not known whether the virus is a new fish virus or a new serotype of SVC virus. It has the same shape, measurements, projections and transverse striations as SVC (ref. 3 and R. Scherrer, personal communication). We have also shown that the SVC virus infects pike and

Table 1 Summary of Experiments on Infection of Pike

No. of experiment*	Dose per fish (p.f.u.)	Water temperature ($^\circ\text{C}$)	Size of fishes (cm)	Mortality		Virological checking	Pathological checking	
				Infected group	Control		Macroscopical lesions	Histological lesions
1	10^6	+14	5-6	6/15	0/15	+	+	+
2	1.2×10^7	+14	5-6	14/15	0/10	+	+	Not performed
3	1.2×10^7	+21-+24	6-1	24/25	1/25	Not performed	+	Not performed
4	4×10^7	+21 +24	6-1	4/7	0/7	Not performed	+	+

* Experiments 1 and 2 were carried out in France; experiments 3 and 4 were carried out in the Netherlands.

produces histological lesions of red disease but in a less acute form than pike virus. In FHM cells, both SVC and pike viruses induce the same CPE and their yields readily increase to high titres (2×10^8 p.f.u. ml.⁻¹) within 24 h at +20° C with an original virus input of 0.1 p.f.u. per cell. Although SVC virus antiserum did not neutralize the pike virus, it is possible that the two viruses are serologically distinct strains of the same virus.

The isolation of rhabdovirus from a pond fish other than carp is very important for fish pathologists. The role of viruses in the occurrence of acute red disease in ponds is still a controversial subject with numerous authors in Germany and Eastern Europe, even for SVC virus. The demonstration of the existence of another virus in pond fishes emphasizes that viruses could have a much more important role than previously envisaged in fish pathology. These observations will have to be taken into consideration in defining disease control measures in pond farming pathology.

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Environmental Modification of the Visual Cortex and the Neural Basis of Learning and Memory

In the primary visual cortex of a normal cat the neurones are almost all orientation detectors, which respond only if an edge or bar at the appropriate angle moves across the receptive field: all orientations are equally represented¹. But early experience can grossly modify the visual cortex. Blakemore and Cooper² reared two kittens in the dark except for a few hours each day spent in cylindrical chambers painted with black and white stripes at one orientation. These two animals, which experienced almost 300 h of exposure to stripes during their first 5 months of life, had no cortical neurones responding to the orientation perpendicular to the stripes that they saw when they were young. Almost all their cortical cells preferred orientations within 45 deg of the experienced angle. The phenomenon involves active modification of the receptive fields of the neurones, and not mere degeneration of unused cells, because there were no regions of silent cortex and no histological evidence of degeneration.

There is a brief sensitive period from about 3–14 weeks of age for this environmental modification, with particular sensitivity during the fourth and fifth weeks³. But in all

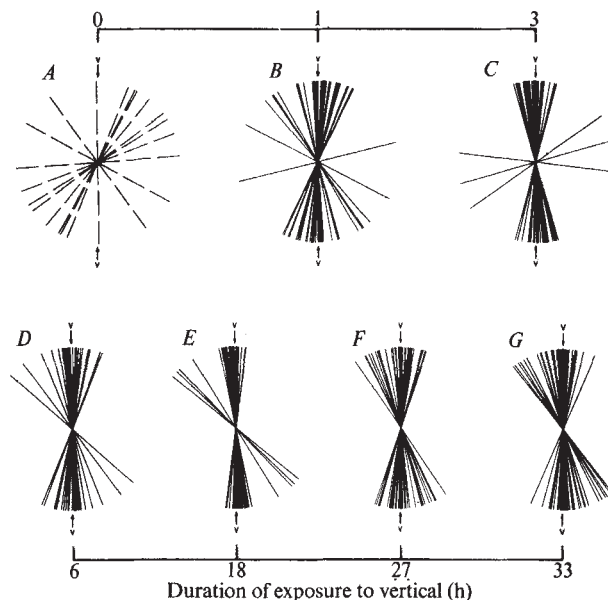


Fig. 1 Diagrams summarizing the effects on the visual cortex of different lengths of time spent in an environment of vertical stripes. Each line represents the optimal orientation for one cortical neurone: for the binocularly deprived animal (A) the lines are interrupted to indicate that these neurones showed only the slightest preference for the angle shown and also responded to all other orientations. The number of hours of exposure for each kitten is indicated on the abscissae.

these experiments the duration of exposure was typically about 30–50 h. One could argue that the whole paradigm is so unnatural that environmental modification can have nothing to do with the genesis of a functional visual cortex in a normal kitten, looking at a normal visual world.

We now know that the process can occur in a very short time. We kept six kittens in the dark from before the time of natural eye-opening until they were about six weeks old, when we recorded from the visual cortex. During the recording the kitten was paralysed with an infusion of gallamine triethiodide and anaesthetized by artificial ventilation with a mixture of nitrous oxide and oxygen. We took the usual precautions to preserve good optical quality and plotted the receptive fields, through each eye in turn, by projecting moving or flashing spots, bars and edges on to a screen 57 cm in front of the kitten's eyes.

Table 1 Visual Experience of the Kittens

Duration of exposure to vertical (h) at various ages														Total (h)
Age (days):	24	25	26	27	28	29	30	31	32	33	34	35	36	
<i>B</i>					1									1
<i>C</i>					1	1	1							3
<i>D</i>					2	2	2							6
<i>E</i>		2	2	2	2	2	2	2	2	2				18
<i>F</i>		3	3	3	3	3	3	3	3	3				27
<i>G</i>		3	3	3	2	3	3	3	2	2	2	3	2	33

A No patterned vision: binocularly deprived.

0

Knowing that the 28th day is a time of peak sensitivity, we exposed these six kittens in a vertically striped cylinder for totals of 1, 3, 6, 18, 27 and 33 h, on or around the 28th day (Table 1). This was the only vision they experienced, except for a few seconds of dim red light from a flashlight each day, during feeding and cleaning. A seventh kitten served as a control animal with no visual experience: both eyelids were sutured before natural eye-opening and the kitten was kept in a normal lighted room. We reopened the lids only just before recording from the cortex.

The polar diagrams of Fig. 1 show the results for all seven animals. In these plots each line is the preferred

orientation for one neurone. The lines are shown interrupted for the zero-hour, binocularly-deprived kitten (Fig. 1A) because we were unable to find any neurone that had more than the vaguest orientational preference. In this animal there were large numbers of visually-unresponsive cells and cells with no orientational preference whatever. Even the neurones illustrated in Fig. 1A responded a little to all orientations and had only the slightest preference for the angles shown. Thus we find ourselves in agreement with Barlow and Pettigrew⁴, who report this pattern as the typical organization of the visually inexperienced cortex, and somewhat at odds with Wiesel and Hubel⁵, who maintain that many neurones in such an animal are wholly adult in their properties.

The visual cortex of even the kitten exposed for 1 h (Fig. 1B) was completely transformed: virtually every cell responded briskly over a narrow range of orientation and the distribution of preferred orientations was strongly biased towards vertical. The few cells maximally sensitive to other angles generally had more infantile properties, such as broad orientational tuning, sluggish responses and rapid habituation. We found them, along with a few non-oriented and visually unresponsive cells, in small regions between the totally normal columns of neurones preferring vertical or near-vertical edges.

For successively longer exposures (Fig. 1C-G) there was a further slight improvement in the narrowness of distribution of optimal orientations and a decreasing probability of finding any infantile neurones. But the difference between 0 and 1 h of vision was much more striking than the final refinements between 1 and 33 h.

The analogy between this process and the ill-understood basis of learning and memory is now no longer far-fetched. We have no idea what fundamental structural transformation in the brain is the physical manifestation of a memory, but it may be very similar or identical to environmental modification of cortical cells. If we can understand this relatively simple phenomenon, perhaps we shall be closer to solving the mystery of memory.

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Defects of Non-Verbal Auditory Perception in Children with Developmental Aphasia

SOME otherwise normal children fail to learn to speak and are designated developmental aphasics. Several authors have suggested that auditory perceptual deficits, particularly of sequencing, may be the primary dysfunction¹⁻⁴. Efron⁵ suggested that the left temporal lobe mediates temporal analysis and that it is the disruption of this function which is central to adult aphasia. We examined children with developmental

aphasia and demonstrated inferior discrimination of sound quality to which a sequencing difficulty could be secondary.

The subjects were nine boys and three girls, aged 6.8 to 9.2 years, from the John Horniman School for Aphasic Children and twelve normal children matched for age, sex and non-verbal intelligence. The aphasics were free from other signs of neurological dysfunction, had normal hearing, and a mean non-verbal IQ of 108 (85-127).

Two different sounds were presented sequentially and subjects were examined for their ability to indicate (a) the order in which the sounds occurred or (b) whether the two sounds were the same or different. Stimuli were two 75 ms complex tones of frequencies within the speech range (fundamental frequency: Tone 1=54 Hz, Tone 2=180 Hz; rise/fall time 40 μ s⁻¹). Normal speech rates approach 80 ms per phoneme. Stimuli were generated by a 'Speech Synthesizer' and 'Modular One' computer, recorded onto a 'Uher' tape recorder and played to subjects through earphones. Subjects responded on two identical depressible panels.

We used two test methods. In the repetition method subjects were trained to respond to each tone separately by pushing the left panel to Tone 1 and the right to Tone 2. Discrimination training continued until criterion was reached (20 of 24 consecutive responses correct, $P<0.001$ binomial test). Subjects were then trained to respond to each of the four possible two-tone sequences (1-1, 1-2, 2-1, 2-2) by pushing the panels in the corresponding order. The inter-sound-interval (ISI) was 428 ms. Correct responses were demonstrated four times by the experimenter followed by eight training trials in which subjects received knowledge of results and error correction. Next, 24 trials without knowledge of results were given. Subjects were then tested on a total of 48 two-tone sequences with ISIs of 8, 15, 30, 60, 150, 305, 947, 1,466, 1,985, 3,023, 3,543 and 4,062 ms, all presented in random order. A similar procedure was used with the same two tones for sequences of three and four elements (ISI 428 ms).

Because a subject may perceive the elements of a temporal sequence but not be able to reproduce a corresponding motor pattern, a same-different method was also used⁶. The response panel was turned through 90° to avoid confusion between methods. Subjects were initially presented with the two tones (ISI 428 ms) and trained to press the top panel if the tones were the same and the bottom panel if different. Training continued until the criterion described above was reached.

The same series of two-tone sequences, 24 with ISI 428 ms and 48 with ISI varied as above, were again presented and the subject indicated whether the two tones were the same or different. Half of the subjects in each group performed the repetition task first; half performed the same-different task first.

On the repetition task, all the aphasics and controls were able to reach criterion both on the initial auditory discrimination and on the reproduction of the two tones separated by 428 ms. However, when the interval between the two tones was decreased, the aphasics' performance was markedly impaired (Table 1). On the repetition test, whereas the controls as a group performed significantly better than chance ($P<0.001$) at the shortest ISI (8 ms), this level of performance was not achieved by the aphasics at ISIs less than 305 ms. By contrast, increasing the ISI had no adverse effect on aphasics' performance. They performed with undiminished efficiency ($P<0.001$) when the interval was increased up to 4 s. When the number of elements in the sequence was increased to three, only two of the aphasics reached criterion. All controls reached criterion on both three and four element sequences.

Results for the same-different task showed a similar pattern. Both groups performed accurately ($P<0.001$) at 428 ms ISI but, when the interval between tones was decreased, controls remained virtually errorless at all intervals whilst the aphasics again required at least 305 ms to perform at the level of accuracy defined above.

Correct performance on the same-different task requires discrimination of the two tones but not identification of the

Table 1 Judgment of Two-tone Sequences by Normal and Aphasic Children

Inter-stimulus Interval (ms)	Repetition method correct %		Same-different method correct %	
	Normal	Aphasic	Normal	Aphasic
8	87*	61	83*	71
15	94*	61	92*	71
30	92*	64	98*	65
60	93*	64	98*	63
150	92*	58	100*	71
305	100*	76*	100*	90*
428	100*	94*	100*	98*
947	100*	94*	100*	98*
1,466	100*	97*	100*	96*
1,985	100*	93*	98*	98*
3,023	100*	98*	98*	100*
3,543	100*	98*	100*	100*
4,062	100*	97*	100*	94*

* $P < 0.001$ binomial test (66% for repetition method; 73% for same-different method).

Percentages are based on totalled scores for each group.

order in which they occur. The ISI at which the aphasics fail to reach the criterion of $P < 0.001$ on a binomial test is the same for both the repetition method (which does require perception of order) and the same-different method (which does not). Accordingly, Efron's and Lowe and Campbell's observations that aphasics exhibit impairment of auditory perception of rapidly presented sequences may be explicable in terms of a failure of discrimination of auditory quality.

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Receptive Fields of the Visual System of the Squid

IN an octopus some second order visual cells that receive synapses in the plexiform layer from the optic nerve fibres have dendritic fields with an oval orientation lying in the horizontal plane¹. It has now been shown that in a squid there are cells with several distinct types of receptive field, which are suitable to act as detectors of visual contours of differing size, shape and orientation. The largest have oval dendritic fields, several millimetres long, predominantly orientated along the vertical (dorso-ventral) axis of the optic lobe (Figs. 1 and 2). Each makes contact with the endings of many thousands of optic nerve fibres and therefore they are probably activated only by very large visual contours. Their large axons reach deep into the optic lobe, from which the axons of third order cells reach direct to the first order giant cells. This may therefore be the pathway for escape reactions from large objects, perhaps also for schooling responses to other squids. The cell bodies of these second order visual neurones lie at the base of the inner granule cell layer and their

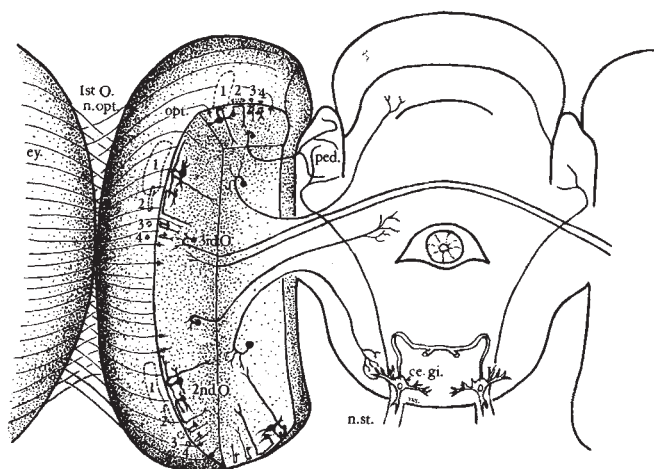


Fig. 1 Diagram of the four types of second order neurone seen in the optic lobe of *Loligo*. The fields of their dendrites are outlined on the surface and numbered 1-4 in decreasing order of size. Only a few further connexions are shown. ey., eye; ce. g., giant cell; opt., optic lobe; ped., peduncle lobe; n. st., static nerve; 1st O. n. opt., fibres of the optic nerves; 2nd and 3rd O., second and third order visual neurones; 1-4, receptive fields of the second order cells.

trunks bend over in the second tangential layer of the plexiform zone to make recurrent axons (Figs. 1 and 3).

Much more numerous second order visual cells have long but very narrow dendritic fields in the first tangential plexiform layer. Their receptive dendrites are short collaterals spread along a single trunk (Figs. 4 and 5). The trunks proceed in various directions over the surface of the lobe, but most are orientated along the vertical axis. Each of these cells is presumably activated only by a narrow visual contour. The whole web of them provides a system capable of responding differentially to complex visual configurations. Numerous amacrine cells among the visual nerve endings provide possibilities of lateral interaction and inhibition (Fig. 4).

The neurones with elongated receptive fields also have their cell bodies in the inner granule cell layer and their axons turn down at the end of the elongated "dendritic" section (Figs. 4 and 5). They proceed to the more superficial levels of the optic lobe where they break up among the processes of numerous small multipolar cells (Fig. 5). This system presumably allows appropriate interactions between the activities of the second order visual cells and signals are passed on further into the lobe by third order cells (Fig. 1).

Besides the neurones with orientated receptive fields there are at least two sorts with circular dendritic fields of various sizes just above the first tangential layer (Figs. 5 and 6). They presumably have circular visual fields. The smallest are in contact with only a very small number of optic nerve fibres, perhaps each with only one; their axons turn inwards and break up near the surface of the lobe.

The possibility of identifying these various types of cell has depended on successful Golgi impregnation in squids. There is little doubt that similar types of cell exist in octopuses and cuttlefishes. The different types of receptive field are largely segregated in the different layers of the plexiform zone. This layering is similar in octopods and decapods, but the former lack the largest cells.

The analysis shows that the plexiform layer of cephalopods provides a system of feature detectors for classifying and encoding the visual input. The categories of visual field suggested by the various types of dendritic field have some obvious similarities with those found by Maturana *et al.*² in the frog or by Hubel and Wiesel³ in the visual cortex of mammals. It will be interesting to discover whether such visual fields can be found experimentally in cephalopods.

At the stage beyond the plexiform zone the integration of

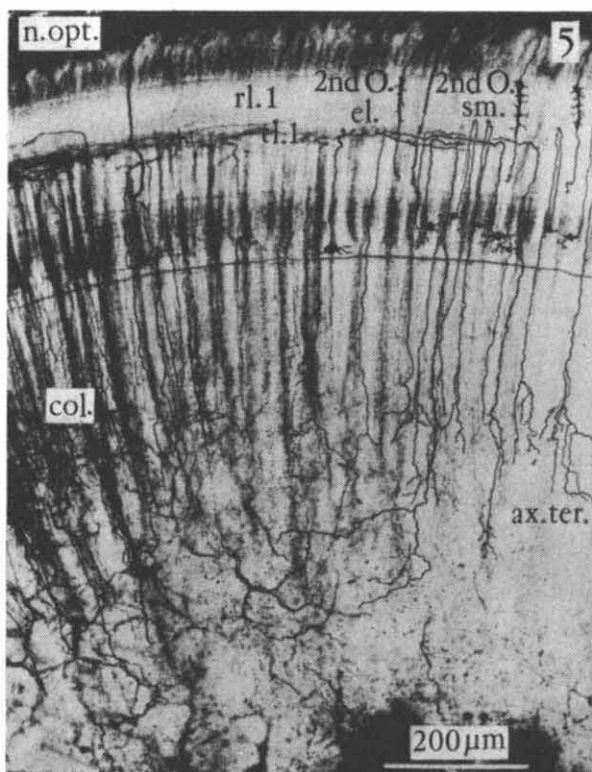
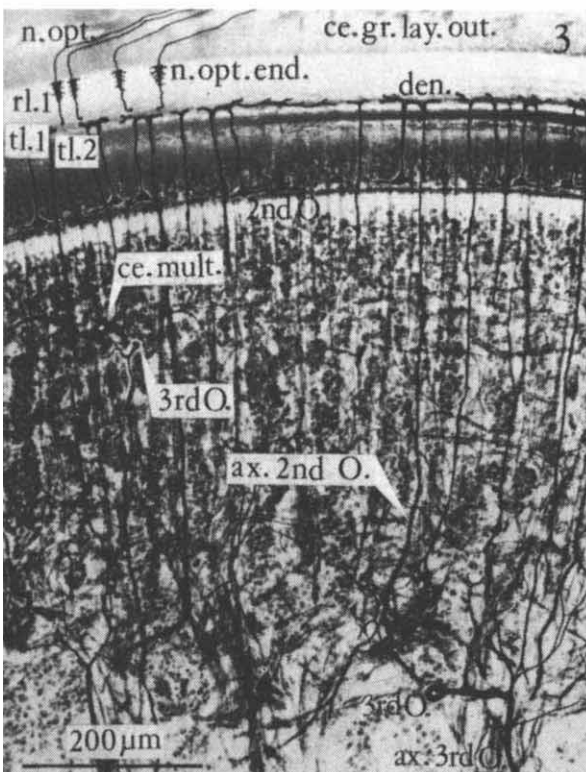
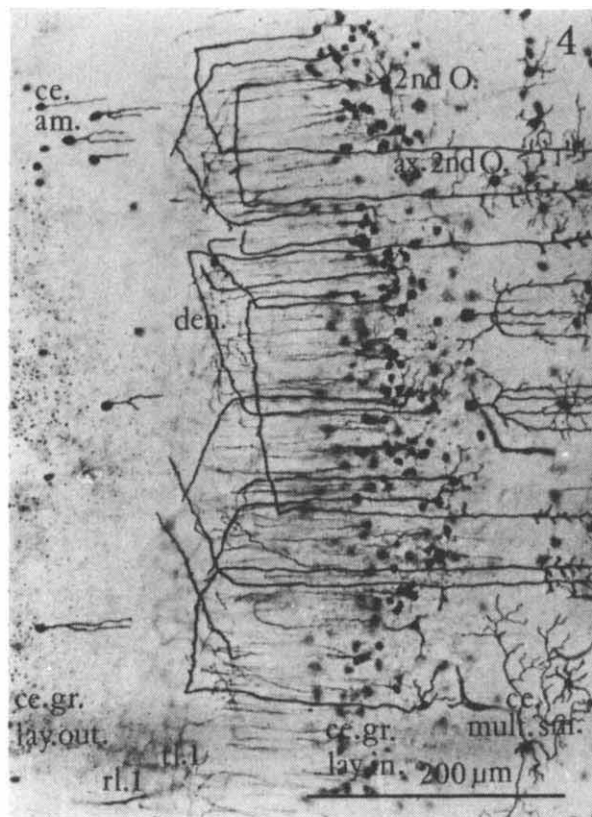
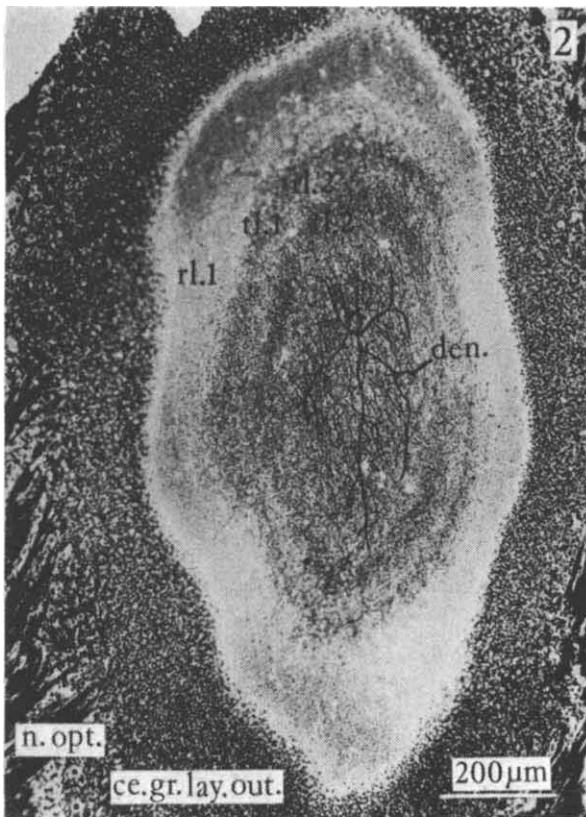


Fig. 2 Sagittal section tangential to the surface of the optic lobe, showing the various layers and the dendritic spread of one large second order visual neurone (den.). Cajal's stain. This and the following figures have been retouched. n. opt., optic nerves; ce. gr. lay. out., outer granule cell layer; rl. 1, rl. 2, radial and tl. 1, tl. 2, tangential layers of the plexiform zone.

Fig. 3 Transverse section to show the largest second order visual neurones (2nd O.) and two third order cells (3rd O.). Cajal's stain. ce. mult., large multipolar horizontal cell; n. opt. end., optic nerve ending; ax. 2nd O., second order cell axon; ax. 3rd O., third order cell axon.

Fig. 4 Second order visual cells with long straight receptive fields (den.) at various orientations, with axons proceeding to the medulla. Also amacrine (ce. am.) and small multipolar cells (ce. mult. sm.) and inner granule cell layer (ce. gr. lay. in.). Golgi-Kopsch.

Fig. 5 Second order visual cells with long straight receptive fields (2nd O. el.) and others with very small fields (2nd O. sm.) at right. The axons form columns in the outer part of the medulla (col.) and terminate there. Golgi-Kopsch.

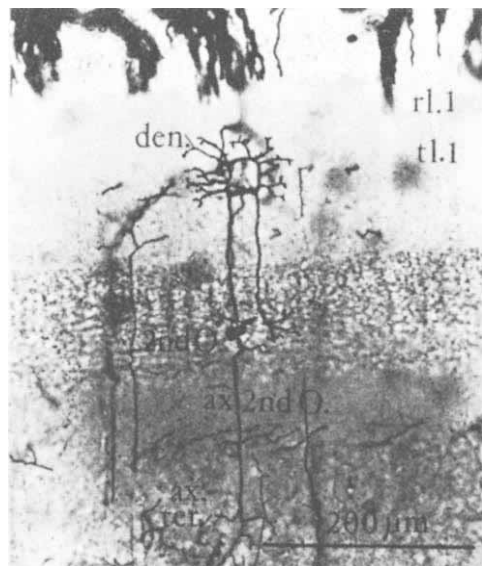


Fig. 6 Second order visual cell with a circular dendritic field lying in and above the first tangential layer. Golgi-Kopsch.

the actions of the various classifying cells is achieved by a system of columns linked by layers of tangentially spreading cells (Figs. 3 and 5). This must be the system by which selection is made of the response appropriate to each visual pattern. Signals are then sent on to the lobes of the central brain by visual neurones of a third or higher order. This system of columns has a certain similarity to the cerebral cortex of mammals, but its mode of functioning remains to be investigated.

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Anodic Oxidation of Urea and an Electrochemical Approach to De-ureation

ONE of the major functions of an artificial kidney is the removal and excretion of nitrogenous waste products. A newer type of artificial kidney (REDY)¹⁻³ converts the urea to ammonia by the action of encapsulated urease. For elimination, the toxic ammonia must then be adsorbed. This device uses a conventional type of dialyser with a recirculating regenerative dialysate system, which does not need large quantities of dialysis fluid. As the dialysis membrane will not filter bacteria, the system can be filled with a small volume of tap water. In practice, a multitude of unwanted waste materials are adsorbed on an activated carbon cartridge, and the urea is converted to ammonia which is then adsorbed on zirconium phosphate. The phosphate

concentration of the dialysate is controlled with zirconium oxide; unfortunately the zirconium compounds are expensive, thus one of the major potential advantages of the system, low cost in use, is not achieved.

One approach to the artificial kidney could be the electrochemical degradation of the nitrogenous wastes and their conversion to non-toxic products, capable of excretion by other means. For example, if nitrogen containing wastes were converted to water, carbon dioxide and nitrogen, the water would be absorbed into the body water pool and the CO₂ and N₂ could be excreted by the lungs. An electrochemical device could conceivably oxidize many of the substances^{4,5} which the kidney normally needs to handle, however, the amount of urea produced by the body requires that any artificial kidney be able to handle large amounts of this substance.

From experiments in this laboratory we conclude that urea may be oxidized electrochemically without prior enzymatic hydrolysis to NH₃ and CO₂. The end products could be N₂, CO₂ and H₂O. These experiments involve (1) linear potential scans of ammonium chloride, urea and glucose plus urea in phosphate buffer solutions and (2) anodic oxidation of glucose-urea in bicarbonate buffer in extra corporeal electrochemical cells.

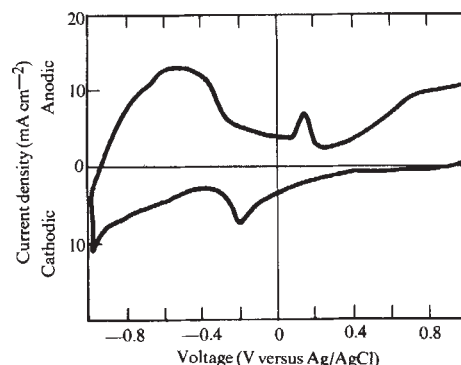


Fig. 1 Potential/current scan of 2.0 M urea in phosphate buffer (pH 7.4) on a Pt electrode.

An 'Electrochemistry System Model 170' (Princeton Applied Research Corp.) was used for the linear potential scanning studies. The system is similar to that used previously⁶. The working electrode was a smooth platinum disk, 1 cm². An Ag/AgCl reference electrode was used. The counter-electrode was a 4 cm² platinum black (40 mg Pt cm⁻²). The electrolyte was Krebs-Ringer phosphate buffer at pH 7.4. Triangular potential sweeps were conducted over a range of -1.00 V to +1.00 V versus Ag/AgCl, that is, -0.32 V to +1.68 V versus RHE (Reversible Hydrogen Electrode potential at the pH of the solution), at a scan rate of 0.20 V s⁻¹. Potential/current curves were recorded on either an oscilloscope or an x-y recorder. The potential sequence used to achieve a well-defined Pt surface followed from that of Giner and Malachuk⁷.

The general character of the scan closely resembles that of similar plots obtained in phosphate buffer by other workers⁷. Fig. 1 shows the cyclic scan recorded for 2.0 M urea in phosphate buffer. A time-variant oxidation peak appears at around 0.12 V versus Ag/AgCl, around 0.80 V versus RHE, in the anodic scans of the urea solution. The same peak appears in the glucose plus urea solution (0.80 M glucose and 2.0 M urea). It is not seen in the scans of both solutions of buffer alone and of NH₄Cl. The chemisorbed species at 0.80 V versus RHE has been tentatively identified as 'reduced CO₂' for the glucose scan⁷ and for scans of other carbonaceous compounds by Giner^{8,9}. We

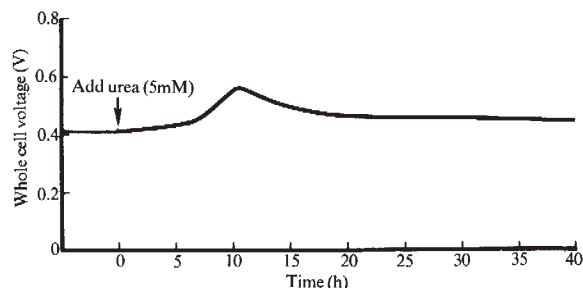


Fig. 2 Effect of added urea on an electrochemical cell operating with 5 mM glucose at the Pt anode, air at the Pt cathode and under 5 Ω load. Initial current density; 82 $\mu\text{A}/\text{cm}^2$.

present here the first evidence that a similar adsorbed "reduced CO_2 " species might be produced in the anodic oxidation of urea.

The presence of this chemisorbed species clearly indicates that urea can be directly oxidized at a Pt anode, possibly yielding CO_2 , NH_4^+ , N_2 and H_2O . Although it would be desirable that NH_4^+ is not a product it also can be oxidized to N_2 and H_2O at a Pt anode under physiological conditions¹⁰. Therefore, we conclude that the final products of anodic oxidation of urea are likely to be CO_2 , N_2 and H_2O .

Experiments on de-ureation by anodic oxidation were carried out in extracorporeal electrochemical cells. A typical cell was made of a sandwich of electrodes and permselective membranes and assembled in a flow-through system^{11,12}. The sandwich consisted of a hydrophilic Pt-black anode, an anion exchange membrane and a hydrophobic Pt-black cathode. The anode was covered with a cellulose membrane and was exposed to a flowing glucose-urea solution or plasma-dialysate (P_{O_2} , 85 mm Hg, P_{CO_2} , 36 mm Hg and at pH 7.4). The cathode was in direct contact with flowing air. Current flow was produced either by shunting the cell with a resistor or by the application of an external d.c. power source. Fig. 2 shows the effect of added urea (5 mM) on an electrochemical cell operating with 5 mM glucose in bicarbonate buffer at the Pt anode, flowing air at the Pt cathode and under 5 Ω passive load. The cell voltage went up from 0.41 V to a maximum at 0.57 V within 12 h following addition of the urea to the glucose solution. Similar voltage enhancement by the effect of addition of plasma or its dialysate, both contain urea, to an operating implantable glucose fuel cell¹² consisting of a Pt anode and a Ag cathode has also been observed and will be reported elsewhere⁵.

These investigations have provided evidence that urea could be anodically oxidized to end products such as CO_2 , N_2 and H_2O . The development of an extracorporeal electrochemical de-ureator which utilizes Pt-black or other catalysts, electricity and air is feasible. This approach eliminates the step of action of urease on urea and the removal of NH_4^+ formed by urease action, by an expensive adsorbent such as zirconium phosphate¹⁻³. The entire renal dialysis system could be made portable.

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Bacterial Identification by Microcalorimetry

FORREST¹ and other workers have shown that interesting data regarding bacterial metabolism can be obtained by microcalorimetry of growing cultures. In our investigations into the use of microcalorimetry as a means of differentiating one microbial species from another, we have obtained characteristic profiles for different members of the family Enterobacteriaceae by observing the heat produced during their growth in liquid media. The microcalorimeter (Instrumentation Laboratory, Inc.) used is of an experimental design operating at 37° C and having a response time of approximately 10 min. The long term baseline stability is 2% full scale or 0.8 $\mu\text{cal s}^{-1} \text{ml}^{-1}$ over a 14 h measuring period. The profiles are recorded on a conventional 10 inch strip chart recorder with a paper feed of 1 inch h^{-1} . The inocula for the temperature equilibrated test vessel consisted of approximately 500 cells obtained by dilution of a logarithmically growing broth culture.

As a broad selection of different organisms was to be studied, a complex culture medium was considered preferable. After tests of several commonly used enrichment media, brain-heart infusion (BHI) was selected. To provide greater cultural uniformity, Baltimore Biological Laboratory lot 01602 BHI was used for all experiments.

Using a sample volume of 4 ml. and observing the growth of the organisms in BHI over periods of 8 to 14 h, we have obtained curves of heat production against time. The complexity of the curves (Fig. 1) should be noted. Forrest¹ showed that heat production ceases abruptly following the exhaustion of available glucose by *Streptococcus faecalis*. Our results show the same phenomenon for that organism in BHI. Sudden changes in heat production occur at many successive points during the growth of some organisms in BHI, as illustrated in Fig. 1. Some peaks are seen which last only a few minutes. Curves for a given organism are virtually superimposable in repetitive experiments. Using these curves we can discriminate between all Enterobacteriaceae yet tested which has included 17 species from 10 genera. *Enterobacter aerogenes* and *Klebsiella*, which are very similar with respect to their behaviour in biochemical tests, are clearly different in respect of their heat production. Some strains of *E. cloacae* can be separated from each other, although each possesses characteristics which permit species identification.

We are presently engaged in a detailed study of the use of microcalorimetry in identification of the organisms encountered in the clinical microbiology laboratory. The evidence indicates

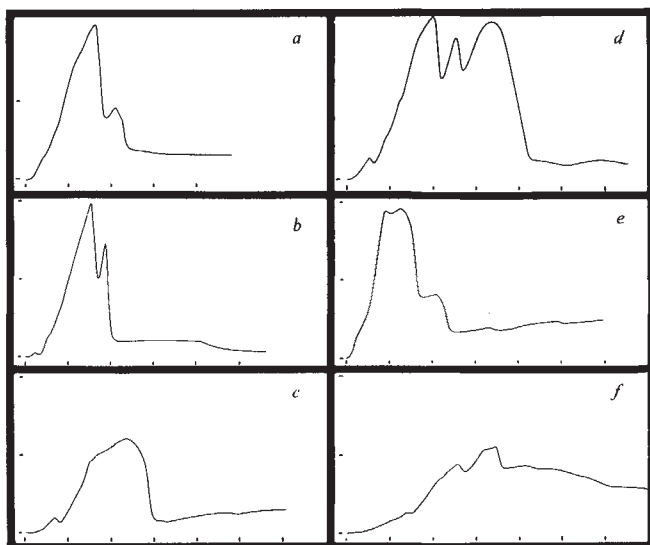


Fig. 1 Six named heat profiles are shown. For each, the abscissa represents time, with 2-h intervals indicated by scale marks, and the ordinate represents heat production, with scale marks at 0, 20, and 40 $\mu\text{cal s}^{-1} \text{ml.}^{-1}$. a, *Enterobacter aerogenes*; b, *Klebsiella*; c, *Proteus vulgaris*; d, *Enterobacter cloacae*; e, *Escherichia coli*; f, *Proteus rettgeri*.

that this approach provides a means for rapid and specific characterization of members of the Enterobacteriaceae. Organisms which do not grow well in broth cultures because of special metabolic needs present problems to be solved. However, for the great bulk of the organisms which must be identified in the routine laboratory, microcalorimetry represents a powerful tool. For research purposes, microcalorimetry provides highly informative data regarding microbial metabolism.

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Nitrosopyrrolidine and Dimethylnitrosamine in Bacon

DIMETHYLNITROSAMINE (DMN) and, in general, many N-nitrosamines are extremely potent carcinogens^{1,2}, and trace amounts of DMN have been demonstrated in certain types of cured meat products and smoked fish³⁻⁵. Certain press reports⁶ in the United States have indicated that significant amounts (up to 0.106 p.p.m.) of nitrosopyrrolidine (NPY) can be formed during the cooking of nitrite-treated cured bacon. Data seem to be very scanty, however, and details have not been published in the scientific literature. Here we provide conclusive evidence for the presence of both DMN and NPY in bacon.

The bacon was purchased from local stores and analysed as such or after frying in an electric pan. The samples were

extracted, and the nitrosamines in the extract purified by a method reported earlier (N. P. Sen, presented at the International Agency for Research on Cancer Meeting on the "Analysis and Formation of Nitrosamines", Heidelberg, 1971). The amount of DMN in the final extract was semi-quantitatively estimated by gas-liquid chromatography (GLC) using a Coulson electrolytic conductivity detector operating in the pyrolytic mode^{7,8}. This detector, under such conditions, is specific for ammonia, amines, and compounds such as nitrosamines that produce these products upon pyrolysis. As free amines and ammonia are removed in the clean-up procedures, very few compounds are present in the final extract that can interfere with the analysis. A level of 0.002 p.p.m. DMN in meat can be detected and the recovery of DMN added to bacon at 0.01–0.02 p.p.m. levels was about 70–80%.

NPY was detected by a specific thin-layer chromatographic technique developed in this laboratory. About 0.1–0.3 ml. of the final extract (1 ml.) was spotted on a silica-gel plate and analysed by the method described earlier⁹. Acetic acid-ninhydrin reagent combination⁹ was used for the final detection. NPY appeared as an orange fluorescent spot when the heated plate was examined inside a 'Chromatoview' chamber (Ultra-Violet Products Inc., San Gabriel, California). Both the short-wave and long-wave transilluminator ultra-violet lamps were used for detection. This technique is very sensitive as well as specific, 50 ng NPY producing a clearly visible spot.

The amount of NPY present in the extract was semi-quantitatively determined by visual comparison of the intensity and size of the spots with that of authentic standards. The detection limit is about 0.005–0.01 p.p.m. of NPY in bacon and other meat products, and the recovery of added NPY (at 0.01 p.p.m. levels) varied between 60–80%. In a few cases, the initial extraction step was omitted and NPY was isolated by direct vacuum distillation of the product in the presence of 3 M potassium hydroxide. This modification resulted in a much better clean-up, and it was possible to detect NPY at 0.002–0.005 p.p.m. levels.

Table 1 Levels* of Nitrosopyrrolidine and Dimethylnitrosamine in Bacon

Sample No.	Kind	NPY p.p.m.	DMN p.p.m.
1	Side bacon, fried	0.02	N.d.†
	Side bacon, uncooked	N.d.	N.d.
2	Side bacon, fried	0.025‡	0.004
	Side bacon, uncooked	N.d.	0.03‡
3	Side bacon, fried	0.01	0.002
4	Sliced bacon, fried	0.025	0.002
5	Sliced bacon, fried	0.01	0.002
6	Sliced bacon, fried	0.004	N.d.
7	Side bacon, fried	N.d.	0.002
8	Side bacon, fried	0.01	0.005

* Uncorrected for percentage recoveries.

† Not detectable.

‡ Results confirmed by GLC-mass spectrometry.

Altogether sixteen samples of bacon (both fried and unfried), five of baby foods containing bacon as one of the ingredients, and six of other meat products were analysed. Eight samples gave a positive test for either DMN or NPY (Table 1); all others were negative. It may be of interest that none of the raw bacon contained any detectable amount of NPY but one contained about 0.03 p.p.m. DMN. Because of its high volatility the level of DMN in this sample fell to about 0.004 p.p.m. on frying. From this limited data it would appear that NPY is formed during frying. As NPY is also fat-soluble the major portion would be expected in

the cooked fat. In one sample (No. 5) about 70% of the total NPy was detected in the cooked fat.

The identity of NPy and DMN from sample No. 2 was confirmed by GLC-mass spectrometry using conditions published previously^{4,8}. The fragmentation patterns of the isolated NPy and that of the authentic standard are very similar (Fig. 1), and both show strong peaks at m/e 100 (M^+) and 30 (NO^+). The isolated DMN produced strong peaks at m/e 74 (M^+) and 30 (NO^+) when analysed by GLC-mass spectrometry using a 'Carbowax' 20 M column⁴, but the mass spectrum showed the presence of some other interfering compounds. Mass spectrometric confirmation was therefore carried out using a second GLC column ('Reoplex' 400)⁴. In view of the positive results with the GLC-mass spectrometric analysis on two columns, and a single peak on GLC analysis with the specific 'Coulson' detector we concluded that the compound was most likely DMN.

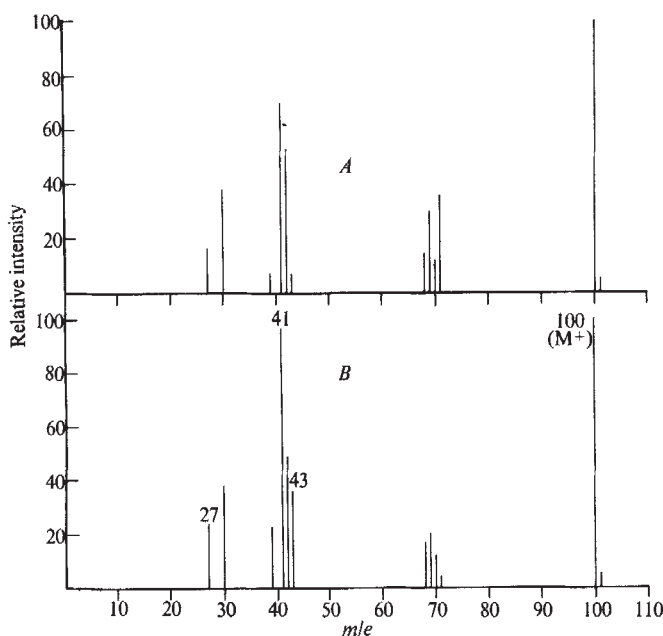


Fig. 1 Mass spectrum of NPy. A, NPy isolated from sample No. 2; B, NPy standard. The background signals (scanned just before the emergency of NPy peak) were subtracted in each case. A 'Hitachi Perkin-Elmer', RMS-4 mass spectrometer coupled with a 'Perkin-Elmer', Model 990, gas chromatograph was used. A 6 foot, 1/8 inch stainless steel column packed with 10% 'Carbowax' 20 M on 60-80 mesh 'Chromosorb' W (HMDS treated) was used for GLC analysis.

Although these values of NPy in bacon are much lower than that (0.030-0.106 p.p.m.)⁶ reported by the US FDA laboratories, both these studies establish that trace amounts of NPy can be present in fried bacon. It is, however, not clear how NPy is formed during cooking. It can be produced in two ways: (a) formation of nitrosoproline from proline and nitrite and subsequent decarboxylation to NPy¹⁰ or (b) by direct interaction of pyrrolidine, which could arise from proline or putrescine¹¹, and nitrite. Further research is needed to establish which of these, or any other reactions, is responsible for the formation of NPy in cooked bacon.

Both DMN and NPy are strong hepatocarcinogens in laboratory animals, although the latter is much less potent¹². In the absence of reliable data on dose-response relationships at low levels it is difficult to assess the hazard to human health arising from the ingestion of trace amounts of nitrosamines in the diet. In view of the extreme carcinogenicity of these compounds in a wide range of species, however, it will be highly desirable to carry out further research which could lead to the control and eventual reduction of the levels of nitrosamines in meat products.

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Natural Inhibitor of the Gypsy Moth Sex Attractant

INHIBITION or masking of sex pheromone response by natural products occurring in the pheromone producing gland has been described in the gypsy moth¹, *Porthetria dispar* (L.), other Lepidoptera^{2,3} and insects in additional orders^{4,5} but none of the inhibitors has been identified. We report here that an olefin, 2-methyl-*cis*-7-octadecene, present⁶ in the female sex pheromone gland of the gypsy moth, inhibits male attraction to the female sex attractant pheromone, which was identified as *cis*-7,8-epoxy-2-methyloctadecane⁶.

Table 1 Mean Catch of Wild Males in Unbaited Traps and in Traps Baited with Attractant and/or Olefin Inhibitor

Treatments	Mean male catch/trap*
10 µg attractant	82.5 a
10 µg attractant + 1 µg inhibitor†	63.5 b
10 µg attractant + 10 µg inhibitor	46.0 c
10 µg attractant + 100 µg inhibitor	5.7 d
10 µg attractant + 1,000 µg inhibitor	8.7 d
Unbaited	0.3 e

* Means followed by the same letter are not significantly different at the 5% level. Treatments replicated four times.

† Olefin inhibitor alone at 1 µg to 1,000 µg attracted <3.0 males/trap.

In field studies conducted in Connecticut, simultaneous evaporation of the attractant and its inhibitor from separate rubber septa wicks (Table 1) in sticky 'Sector' traps ('Model XC-26', 3M Co.) shows that even low release rates of the olefin suppress male attraction, whereas somewhat higher release rates of olefin effectively inhibit attraction. More importantly, at these same release rates the olefin also eliminates the attractiveness of live calling females (Table 2, Experiment 1).

The *raison d'être* for the inhibitory action of the olefin may be due to competition for the same olfactory receptor sites as the attractant on the male antennae. This was suggested as a mechanism for inhibitory effects of certain compounds structurally similar to sex attractant pheromones^{7,8}. Although it seems that the olefin is a precursor to the pheromone⁶ we also considered possibilities for its natural release to control behavioural activity of the males. Females mate only once and mated females usually do not lure males⁹; by releasing the olefin an inseminated female could escape copulatory attempts by males and would thus deposit her eggs unmolested. Our field tests suggest, however, that females release little or no olefin after mating (Table 2, Experiment 2), for although freshly mated (and hence non-calling) females are not attractive, an attractant wick added to traps baited with freshly mated females (Table 2, Experiment 3) is attractive and apparently is unaffected by the mated female.

Table 2 Mean Catch of Wild Males in Sticky Traps Baited with Caged Mated or Unmated Females Compared with Catch in Presence or Absence of Attractant or Olefin Inhibitor

Treatments	Mean male catch/trap
Experiment 1	
Virgin female	44.0 a *
Virgin female + 1,000 µg inhibitor	1.3 b
Experiment 2	
Virgin female	27.7 a *
Freshly mated female	0.5 b
Experiment 3	
Freshly mated female + 10 µg attractant	13.2 a *
10 µg attractant	11.4 a

* Means followed by the same letter are not significantly different at the 1% level. Treatments replicated four times.

The behavioural effects of the olefin indicate that it might be more effective than the attractant in disrupting the gypsy moth communication system in field trials¹⁰ in which areas are flooded with numerous sources of chemical for suppression of this important forest defoliator. The olefin would be particularly effective if it causes mating inhibition at the central nervous system level.

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Growth and Metabolism of *Ostrea edulis* Larvae

ONLY a fraction of the plant food captured by zooplankton is converted into growth; the rest is lost through incomplete assimilation, excretion and metabolism. The net growth efficiencies, K_2 (percentage of assimilated food converted into growth) for haloplanktonic animals such as *Calanus* and related copepod species are generally lower than the percentage of assimilated food lost in metabolism ($100-K_2$). In terms of calories, values of K_2 quoted in a recent review by Corner and Davies¹ range from 23–58 for *C. hyperboreus*, from 6–55 for *C. helgolandicus*, and from 14–29 for *Acartia clausi*. There are few comparable estimates of growth efficiencies for meroplankton species, such as molluscan larvae, but the data that does exist suggests that the values of K_2 are considerably higher than those for copepods. Jørgensen calculated a value for K_2 of 73% for *Mytilus* larvae and values of 62% and 63% for two gastropod veligers², and from short-term feeding experiments with *Ostrea edulis* Walne estimated that 68–80% of the total food assimilated by the larvae is utilized for growth³. During larval development the daily level of assimilated food declined from 56% to 29% of the body weight.

One of us (DLH) has recently published data on the changes in biochemical composition of *O. edulis* larvae during development and on the losses of the body components during short-term starvation experiments⁴. We have calculated an energy budget for *O. edulis* larvae from this data in terms of growth and metabolism.

Three batches of larvae, released between February and April, 1971, were reared at the MAFF Fisheries Experiment Station, Conway. The larvae were kept at 20–22° C in aerated seawater, and fed a mixed algal diet of *Isochrysis galbana* and *Tetraselmis suecica*. On release (day 0) and subsequently on days 4, 8 and 12, samples of approximately 10–15,000 larvae were collected for biochemical analysis at the NERC Unit of Marine Invertebrate Biology, Menai Bridge. The larvae were counted and analysed for protein, lipid and carbohydrate content as described previously⁵. Further samples of larvae were taken from the main culture vessels, on the days indicated, and placed without food in aerated seawater for 48 h before biochemical analysis.

Table 1 Protein, Lipid and Carbohydrate Content (mg per 10⁶ larvae) of *Ostrea edulis* Larvae during Development

Days after release	Length (µm)	Protein	Lipid	Carbohydrate	Total calories
0	179.3	118.3	29.8	14.4	1,009
4	221.9	309.1	121.7	42.5	3,070
8	266.2	610.4	249.1	67.3	6,079
12	296.8	1,083.6	543.7	130.2	11,794

Tables 1 and 2 show the biochemical composition of *O. edulis* during development (mean values for the three experiments) and the mean losses of protein, lipid and carbohydrate on starvation. The results are expressed in terms of 10⁶ larvae. *Ostrea edulis* larvae can survive for several days without food. During this time the necessary energy is derived from reserves in the body tissues and the total energy loss, during starvation, can be taken as an

estimate of the metabolic energy demand. Conventionally metabolic rates are calculated from measurements of oxygen consumption during respiration. There is reasonably good agreement between the loss of body reserves and experimentally determined rates of oxygen consumption for newly released *O. edulis* larvae⁶. This need not necessarily be true however, as for example, when there is a partial anaerobic metabolism. As a first approximation we have used the data from the starvation experiments to calculate metabolic rates for *O. edulis* larvae.

Table 2 *Ostrea edulis*. Loss of Protein, Lipid and Carbohydrate (mg per 10⁶ Larvae) During Starvation for Two Days

Days after release	Protein	Lipid	Carbohydrate	Total calories
0	10.2	8.1	1.4	140
4	20.2	45.0	10.4	582
8	74.9	74.5	4.2	1,144

On release (days 0–2) the daily loss of body reserves is equivalent to an oxygen consumption rate of 0.6 $\mu\text{l. } 10^{-3} \text{ O}_2$ per larva h^{-1} . This value is close to the value given by Millar and Scott⁶ for newly released larvae kept without food at densities in excess of 100 larvae ml^{-1} . The daily loss of reserves for days 4–6 and 8–10 are equivalent to oxygen consumption rates of 2.5 and 5.0 $\mu\text{l. } 10^{-3} \text{ O}_2$ per larva h^{-1} respectively. The former value is approximately half the rate determined by Walne for larvae of the same size range feeding on *Isochrysis*⁷. Zeuthen concluded from measurements on individual *Mytilus edulis* larvae that the rate of oxygen uptake for “resting” metabolism was approximately half the rate for “swimming” metabolism⁸. In Table 3 we have equated our calculated values for the metabolic energy demand during starvation with resting metabolism and doubled these values to obtain the figures for swimming metabolism.

Table 3 Growth and Metabolism of *Ostrea edulis* Larvae

Days after release	Energy values: calories per 10 ⁶ larvae day ⁻¹			
	Growth	Metabolism Resting	Swimming	Net growth efficiency (K_2)
0–2	515	70	140	78.6
4–6	752	291	582	56.4
6–10	1,429	572	1,144	55.5

The net growth efficiencies K_2 for swimming larvae fell from 78.6% on release to 55.5% on day 10. The daily food requirements (assimilated ration) to support growth and metabolism represented 64.9% and 42.3% of the body weight on day 0 and day 10 respectively.

The values of K_2 are probably conservative estimates because of doubling of the energy loss during starvation to give the level of swimming metabolism. Oyster larvae, for example, when released from the adult can survive for several days without food and can be swimming actively during this time⁶. Based on the data for resting metabolism the corresponding values for K_2 would be 88.0% on day 0 and 71.4% on day 10. Our calculations, therefore, confirm the view that molluscan larvae have high net growth efficiencies^{2,3}, such that 50–80% of the assimilated food is utilized for growth. Whether or not such high values are limited to young, rapidly growing stages needs further study. Corner and Davies¹, quoting data obtained by Petipa from the Black Sea, suggested that K_2 values for young copepodite stages are higher than those for older animals, although in terms of body nitrogen it is the older stages that show the most rapid growth⁹.

Finally, the total energy demand (growth plus metabolism) can be compared with published data on the rate of consumption of *Isochrysis* by oyster larvae at different stages of development³. Walne gives values of 395–600 cells per larva h^{-1} for larvae of mean length 178 μm and 916–1517 cells per larva h^{-1} for larvae of mean length 219 μm . These values can be converted into calories (10^6 cells = 0.016 mg dry matter = 0.09 cal) and used to give an estimate of assimilation efficiencies on day 0 and day 4 respectively. The calculated values for assimilation efficiency range from 50.8–77.1% on release to 40.9–67.7% on day 4. Although it is recognized that these values are necessarily approximate they are considerably higher than the values of 15–45% calculated by Walne³ from feeding experiments with ³²P-labelled algae and are comparable to the values reported for *Calanus*¹.

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Antibody to *Babesia* in Scottish Red Deer (*Cervus elaphus*)

Babesia spp. have a world wide distribution in domestic animals¹; and have also been described in wild animals^{2,3}. The occurrence of this protozoon, however, is poorly known since the organism is rarely detectable in blood films except for a short period soon after initial infection. After this time, if the host survives, the infection becomes latent. Infections may be diagnosed by serological methods. The indirect fluorescent antibody (IFA) test has the practical advantage that it is simple to perform and requires only a small volume of serum; it can also be carried out with blood collected on filter paper.

Babesia spp. of domestic animals are thought to be host specific. Callow⁴, however, has shown that when ticks infected with *B. bigemina* were allowed to feed on sheep, viable parasites appeared in the blood. When blood was transferred from sheep into susceptible calves, an obvious infection with *B. bigemina* developed.

It has also been shown that when *B. divergens* and *B. motasi* were introduced by blood inoculation into splenectomized red deer, *B. divergens* persisted for at least 230 days and *B. motasi* for 100 days, in the blood stream^{5,6}. In Scotland, many tick infected pastures are shared by cattle, sheep and red deer. In some areas *B. divergens* has been reported in the cattle, and we thought it would be of interest to look for babesial antibody in red deer.

Normal serum was obtained from deer which had been born and reared at the Rowett Research Institute, Aberdeen. A globulin fraction was prepared by precipitation in 50%

ammonium sulphate. An antideer globulin was obtained by intradermal injection into rabbits of the globulin fraction in Freund's complete adjuvant. The globulin fraction of this serum was separated by ammonium sulphate precipitation and conjugated with fluorescein isothiocyanate (isomer I, Sigma). Excess dye was removed by filtration on a 'Sephadex G-25' column, and non-specific fluorescence was removed by absorption with rabbit liver acetone powder (Sigma).

Sera or blood on filter paper from 25 deer shot in Ross-shire and Inverness-shire and blood samples from Arran were examined. The IFA test was performed as described by Brocklesby *et al.*⁷ with *B. divergens* as the antigen.

The sera of 7 out of the 25 mainland deer had babesial antibody; the titres of the sera ranged from 1/40 to 1/320. One of the 2 blood samples from the Arran deer fluoresced brightly when the antigen was treated with eluate from the filter paper. Fluorescence was absent when the antigen was treated with normal deer serum at dilutions greater than 1/10. The titres of the deer sera were similar to those found in a survey for babesiasis in cattle on the island of Arran⁸.

The results indicate that red deer in Scotland are parasitized by a *Babesia* sp. We believe that this is the first record of this parasite in red deer in Britain⁹, although *Babesia* has been found in deer in other parts of the world^{10,11}.

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Chimpanzee Tool Use in Dental Grooming

Tool use by animals is characteristically restricted to agonistic, exploratory, or self-maintenance contexts, and we have only one record of its use in social grooming¹. Belle, a female chimpanzee (*Pan troglodytes*) at the Delta Regional Primate Research Center, used sticks as aids in grooming the teeth of other individuals, and on one occasion she fashioned a stick tool by stripping a twig of its leaves. In 135 h of observation over 6 weeks, we recorded forty-five bouts of dental grooming: four of tool use (all social), twenty-three of social grooming, seven of attempted social grooming, and eleven of self grooming. Dental grooming occurred on 16 of the 27 observation days.

The main cause was probably Bandit's (the youngest male) shedding of deciduous molars. His teeth had two fresh vacancies and one loose movable tooth barely in place. In some cases



Fig. 1 Belle (sitting) dental grooms the teeth of Bandit (lying supine) with a small pine twig tool. Shadow (standing bipedally) looks on.

Belle definitely concentrated her efforts on these sites.

Seven adolescent chimpanzees (aged 7–11 yr) lived as a group for 3.5 years in a one acre outdoor enclosure carpeted with natural herbaceous vegetation and containing over seventy upright or fallen poles and tree trunks. Fresh branches and saplings were introduced weekly. We observed the group's activities from an overhead observation deck using binoculars and a camera with a telephoto lens.

In all four instances of tool use in dental grooming, Belle was the "dentist" and Bandit the "patient" (Fig. 1). Her usual procedure was to begin normal social grooming directed to the hair of his limbs, torso, or head. She then concentrated on his face and, finally, opened his mouth. If he cooperated by gaping his jaws, she began dental grooming with the fingers, accompanying this with "teeth clapping", a sound produced during high intensity grooming by rapidly banging the teeth together. Sticks used as supplementary aids were picked up from the surrounding substrate, apparently on the spur of the moment. The single case of tool modification occurred on the day after red cedar (*Juniperus virginiana*) branches had been introduced, and the area around the grooming pair was littered with fragments (see background of Fig. 1). Belle picked up a twig approximately 0.5 cm in diameter and stripped off the leaves, leaving a pencil-like object about 15 cm long. Between sessions of using it as a pick, she left it hanging loosely out of the corner of Bandit's mouth, or held it between her own lips like a cigarette, or rested it on his chest if he were lying supine. Several tools have been recovered (Fig. 2), and all have scraping or probing edges. The tool-using grooming bouts were protracted: 730, 530, 300, 185 s in length. Ordinary social grooming bouts averaged 114 s in duration ($N=202$, range: 5–1,779). Following dental grooming, Belle returned to normal grooming of other body parts.

Belle was also responsible for twenty-two of the twenty-three dental social grooming bouts without tool use. Bandit received eighteen of these, and Shadow (another male) received four. Her general procedure resembled that already described. She commonly used her thumbs as scrapers and her forefingers to pick particles loose. These were rapidly flicked out of the mouth, or, more uncommonly, the two forefingers were used together as forceps to remove the particle. She apparently concentrated picking on molars and scraping on incisors. She seemed to be equally dextrous with either hand, and she sometimes supplemented her hands by using her lips. Her usual practice involved using one hand to steady the patient's head while working with the other. When grooming the lower teeth, she hooked the opposite-hand thumb under the upper jaw and spread the fingers over the forehead. When working on the upper teeth, she held the lower jaw maximally gaped with the non-working hand. Belle adopted a variety of postures while working: sitting, bipedal standing, crouching, reclining. The patient usually lay supine, but Belle manoeuvred him through other postures. Dental social grooming bouts not

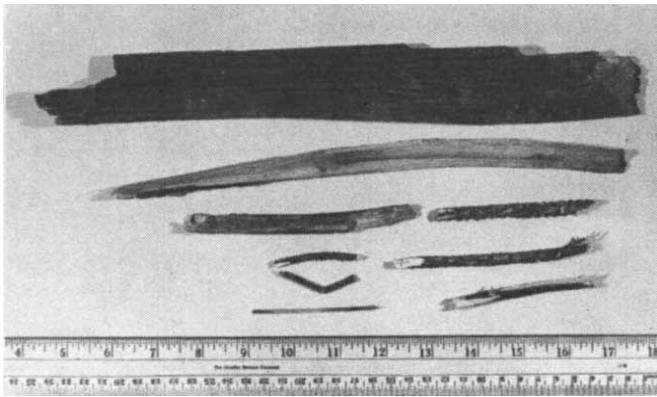


Fig. 2 Range of wooden tools used by Belle in social dental grooming.

involving tools averaged 56 s in length ($N=18$, range: 10–283). These were significantly shorter than dental grooming bouts involving tools (Mann-Whitney U test, $U=1$, $P>0.002$, two-tailed).

Belle also exhibited all the unsuccessful attempts at dental grooming and directed these to four different individuals. They followed a consistent sequence: Belle groomed another chimpanzee normally, then switched her manipulations to the groomed animal's mouth. The latter compressed its lips and turned away its head (and in one extreme case, covered the face with the forearms). This prevented further dental grooming and Belle returned to normal grooming. In one instance, however, she responded to Bandit's refusal to be dentally groomed by flipping his lower lip down. This everted it over his chin, and she continued dental grooming in spite of his clenched teeth. Her unsuccessful attempts were of short duration, the longest lasting 13 s.

We saw six of the seven chimpanzees groom their own teeth, and no single individual exhibited a disproportionate share of the group's total. The thumb was usually used to groom the upper teeth and the forefinger used to groom the lower teeth. We saw no self grooming tool use, but wild chimpanzees commonly use tools in grooming their external body surfaces. They remove sticky substances by wiping with leaves and branches, and water by rubbing against tree trunks. Only great apes have been seen to use tools in self grooming of body orifices: van Lawick-Goodall presented one case each of chimpanzee self grooming of the teeth and nose with twigs². Captive chimpanzees may use mirrors as visual aids to pick at their own teeth and nostrils³.

Apart from its hygienic function, primate social grooming is the most common socially affiliative behaviour, involving prolonged intimate physical contact, and it presumably reinforces social bonds and thereby has ramifications in social organization and structure^{4,5}. Miles inferred the presence of altruistic intentions in grooming to relieve the discomfort of afflicted individuals⁶. Yet only once before has the use of tools been recorded in this important primate activity. Van Lawick-Goodall saw a wild female chimpanzee use leaves to wipe sticky banana from the head of her infant sibling².

Recent observations have re-emphasized the chimpanzee's paramount position as a maker and user of tools amongst the non-human primates^{7–11}. Yet the dental grooming reported here is the first systematic use of tools in friendly social interaction to be recorded. Of course, many primates are capable of complicated tool use under special laboratory or zoo conditions¹², often intentionally helped by human intervention, such as animal intelligence testing¹³. The spontaneous use of tools described here, however, was shown by animals living a self-regulating, natural life. Obvious antecedents of the activity such as copying human use of tooth picks could be ruled out, and no human reinforcement was given.

Why did this unique form of tool use appear in this group of chimpanzees? All the individuals were wild-born and did not arrive at Delta until at least 1–4 yr old. Previous comparisons of wild and captive born chimpanzees have shown the former's superiority in the use of tools¹⁴. For most of their stay at Delta the chimpanzees lived in a large outdoor enclosure containing innumerable movable objects up to the size of telephone poles, and many novel objects were introduced over the years. Because the overall group of chimpanzees or sub-groups of it were housed in mutual access from infancy, they had intimate, well-socialized relationships. This was exemplified in the broad latitude allowed each other in grooming. In addition to grooming each other's hairy body surfaces, they groomed each other's eyes, nostrils, ears, genitals and anuses.

Belle performed 97% of the social dental grooming observed (the exception was performed by a male to another female). She also showed the most frequent ordinary social grooming and the longest mean bout length of any individual in the group, and was one of the two most frequent tool users in other contexts. The other frequent tool user, Bandit, was the principal recipient of Belle's dental efforts, receiving twenty-four bouts. Shadow accounted for five, and two other chimpanzees two each. The subgroup of Belle, Bandit, and Shadow lived in uninterrupted contact from their arrival at Delta in June 1966, giving them the longest shared experience of any of the animals. If any substitute kinship relations existed in this group, this triad (pictured in Fig. 1) was likely to possess them. Belle also directed 54% of her non-dental grooming to Bandit. He was apparently a most groomable individual; he received more grooming than any of the others and had the highest overall partner preference rank in social grooming although he rarely groomed others.

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Menarchal Age in Norway: Halt in the Trend towards Earlier Maturation

STATISTICS on the age at menarche have shown that girls have been maturing progressively earlier in all parts of Europe, North America and Japan during the past fifty years^{1,2}. The rate

of advance of menarche, about 4 to 5 months per decade, has been constant in all countries during this period. There is some evidence that this advance of the onset of puberty can be traced back to the middle of the last century in some countries, among them Norway¹. There have been no published reports of a cessation of this trend, and Tanner² believes that the trend "may be extrapolated onwards for at least another decade or two".

Two different methods have been applied to obtain estimates of the age at menarche³. (1) Girls of the relevant ages have been asked whether or not they have experienced their first period (the *status quo* method). (2) Older girls or women have been asked about their age at their first period (the "recall" method). All information about the age at menarche in the nineteenth century was obtained by the recall method, and probably with very biased samples of the population. The *status quo* method was introduced by Schiøtz⁴ in a study of schoolchildren in Oslo (Kristiania) in 1918, and has generally been accepted as the better of the two methods. Unfortunately, his first investigation involved only 177 girls aged 12 to 17 yr. His statistical calculations gave a mean menarchal age of 15.01 yr. Recalculations from his published tables by the numerical methods described here give a mean age of 14.57 yr and a standard deviation (s.d.) of 1.18 yr. Two large scale investigations, one by Schiøtz⁵ in 1928 and the other by Letting⁶ in 1952, were carried out using the *status quo* method on schoolchildren in Oslo. Each study involved about 10,000 girls 10 to 17 yr old. The published mean ages at menarche were 14.28 and 13.44 yr. Our recalculations give 14.18 yr (s.d.=1.15 yr) and 13.27 yr (s.d.=1.11 yr). In the period 1928 to 1952 the average advance of the menarchal age was 4.5 months per decade, a rate that agrees with results obtained in other countries^{1,2}. Extrapolation of this trend to 1970 would predict a menarchal age of 12.6 yr.

Table 1 Percentage of Menstruating Girls at Each 3 Months of Age

Age group centres (yr)	Number of menstruating girls	Total number of girls	Percentage of menstruating girls
11.00	2	292	0.68
11.25	4	251	1.59
11.50	10	269	3.71
11.75	12	259	4.63
12.00	32	293	10.92
12.25	49	262	18.70
12.50	59	266	22.18
12.75	91	283	32.15
13.00	115	282	40.78
13.25	151	296	51.01
13.50	188	289	65.05
13.75	192	271	70.84
14.00	212	286	74.12
14.25	218	263	82.88
14.50	243	276	88.04
14.75	305	334	91.31
15.00	284	315	90.15
15.25	282	298	94.63
15.50	266	274	97.08
15.75	293	299	97.99
16.00	298	305	97.70
16.25	276	282	97.87
16.50	270	274	98.54
16.75	296	301	98.33
17.00	334	335	99.70
7,155			

Our investigation, carried out in March 1970, included physical measurements in addition to questions about menstruation. Only the results concerning the age at menarche obtained by the *status quo* method will be published here. All primary and high schools in Oslo were included in the investigation; schooling is compulsory in Norway up to and including the age of 16, and 50% of the children continue in school for at least two more years. The results obtained from 7,155 girls are given in Table 1. The percentage of menstruating girls at

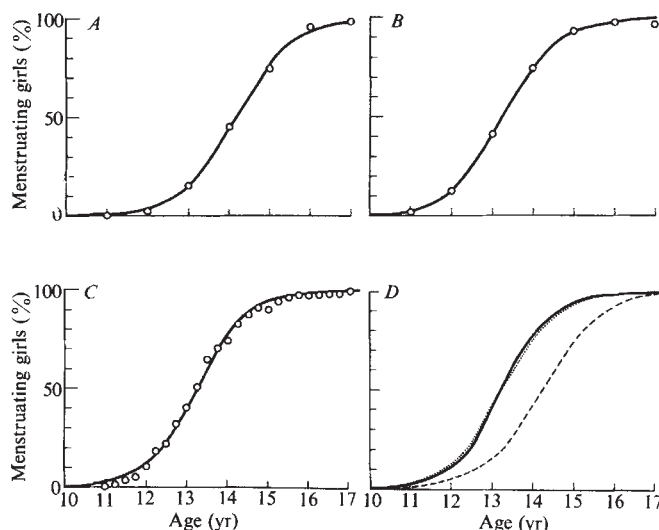


Fig. 1 Cumulative menarche-age distributions. *A*, 1928 empirical data from Schiøtz⁵. Line plotted from best fitted logistic function; $Y=100.0/(1.0+\exp(1.6675 * (\text{Par}(1)-X)/\text{Par}(2)))$ where Y : percentage of menstruating girls at the age X (yr) $\text{Par}(1)$: mean menarchal age (MMA) (yr); $\text{Par}(2)$: standard deviation (s.d.) (yr), MMA=14.18 yr and s.d.=1.15 yr. *B*, 1952 empirical data from Letting⁶. Line as in *A* with MMA=13.27 yr and s.d.=1.11 yr. *C*, 1970 empirical data from the present investigation. Line as in *A* with MMA=13.24 yr and s.d.=1.04 yr; *D*, The three best fitted theoretical distributions plotted in one figure. ---, The 1928 curve; ..., the 1952 curve; —, the 1970 curve.

each 3 months of age are plotted as a function of the centre of the age group in Fig. 1C. The mean menarchal age and its s.d. were estimated from this empirical cumulative menarchal age distribution by fitting a theoretical curve to it with a standard non-linear least-squares procedure. The theoretical functions were either a cumulative normal distribution calculated by a computer subroutine or a logistic function. Both functions gave identical results. The mean age at menarche in Oslo in 1970 was estimated to be 13.24 yr (s.d.=1.04 yr). The corresponding logistic curve is plotted in Fig. 1C. The empirical data from Schiøtz and Letting's investigations are plotted similarly in Fig. 1A and B with the corresponding best fitted logistic functions.

Although the mean menarchal age decreased from 1928 to 1952, it did not change at all from 1952 to 1970 (Fig. 1D). This result seems to be the first indication in any country of a halt in the trend towards earlier physical maturation. Both the methods used and the populations studied in the three investigations are probably as comparable as they can be in view of the fact that more than forty years elapsed between two of the studies. All investigations were carried out within the Oslo city school health system, originally organized by Schiøtz, and the changes in the city have probably been, in most respects, less than in many cities of comparable size.

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Mental Disorder and Season of Birth

MOST investigations of the relationship between season of birth and mental illness have found that a disproportionate number of patients with schizophrenia or manic-depression were born in the early months of the year^{1,2}. Most of these studies can reasonably be criticized on the grounds that the samples of patients were (a) not representative of all patients, and (b) not comparable with the general population used as the basis of expectation, because seasonal distribution of births in a general population may vary significantly not only from year to year but between adjacent geographical areas in the same year³. Dalen's study⁴ largely avoided these criticisms because his schizophrenic patients were representative of the whole of Sweden; he made a decade-by-decade comparison (P. Dalen, personal communication) of years of birth with the general population. He found a very significant excess of the patients had been born in the months January to March.

Here we report further evidence in support of this, using material we believe to be free from these criticisms. Since 1970, the month as well as year of birth of all persons admitted to psychiatric beds in England and Wales have been recorded, and we used data from all patients first admitted in the years 1970 and 1971 who had been born in England and Wales from 1921. These cases can have no sampling bias and their seasonal distribution of births may properly be compared with the general population of the same geographical area from the year 1921. It was not possible to compare seasonal distributions of month of birth between the two populations before 1939 as it is only from this date that the monthly number of live-births in England and Wales has been published. It was possible, however, to compare seasonal birth distributions in terms of the first, second, third and fourth quarters of the year as these numbers are available for England and Wales from 1921, and the psychiatric admissions in 1970 and 1971 can be regrouped into quarters of the year from the monthly distributions made available to us. The comparisons made by us are therefore undisturbed by any heterogeneities, for example, in variation between urban and rural geographical areas or in variation from year to year, because all these sources of variation are exactly balanced between the two series. The reservations one would have to make to this comprehensive statement are unimportant. The general population cohort born in the year Y will have been reduced due to death and emigration by the year $(Y+x)$ when admissions to hospital occur. Immigration does not enter the picture as persons not born in England and Wales are excluded from the figures. Emigration will have affected the general population cohort by the time in the year $(Y+x)$ when psychiatric morbidity begins to operate, but there is no factual or plausible ground for supposing that emigrants differ in season of birth from the population from which they come. Deaths may, of course, influence the distribution of season of birth in the total population in the years after year Y if, for instance, infants born in one or another season have a higher or lower mortality than others. We consider it exceedingly improbable, however, that there has been any such effect operating on the population as a whole, indifferently of any potentiality to psychiatric morbidity; it can be seen from Table 1 that there is no shift in distribution of season of birth for psychiatric patients as a whole but only for two diagnostic sub-groups, the manic-depressives and the schizophrenics. Only in these two diagnostic groups is a statistically significant shift displayed.

Table 1 shows the observed and expected numbers of births in the first quarter-year for five diagnostic categories. The expected numbers are calculated as follows. If $N_{s(1921)}$ is the total number of schizophrenic patients born in 1921 and admitted to hospitals in the years 1970 and 1971, B_{1921} is the total number of live-births in England and Wales in that year, and F_{1921} is the number of that total born in the first quarter, then the number of schizophrenics, born in 1921, who could be expected to have been born in the first quarter

is $N_{s(1921)} \times F_{1921}/B_{1921}$. All these yearly expectations are summed from year to year to give the total number of expected first-quarter-born schizophrenics for all years up to 1955, so as to provide the schizophrenia total (Table 1). The same procedure was used to calculate the expectations in the other four diagnostic groups. The total expected was then set against the total observed, and one degree of freedom used to estimate the significance of χ^2 .

Table 1 Observed Numbers of Patients* (by Diagnosis), Born in the First Quarter of the Year Compared with Expectation from all Live Births in England and Wales†

Diagnosis	Total patients	Nos. in first quarter Observed	Expected	Ratio O/E	χ^2 (1 d.f.)‡
Schizophrenia	5,139	1,383	1,292.1	1.07	8.55
Manic-depressive psychosis	3,523	961	883.3	1.09	9.13
All other psychoses	2,852	708	713.9	0.99	0.06
Neurosis	12,061	3,056	3,024.2	1.01	0.45
Personality disorder	4,479	1,114	1,127.8	0.99	0.23

* All patients born in England and Wales 1921–55 and first admitted to a psychiatric unit 1970 or 1971.

† Sum of year-by-year comparison.

‡ First quarter and the remaining part of the year.

Table 1 shows that for schizophrenia and for manic-depressive psychosis there is a significant excess of births in January to March ($P < 0.01$). The observed numbers in the other diagnostic groups differ only very slightly from expectation. The trend for each diagnosis is the same when the two years of admission are considered separately.

We think these results are important because they add substantially to the evidence for a real association between season of birth and functional psychosis. If real, this is the first association to be demonstrated between the incidence of schizophrenia and a clear-cut, objectively measurable, environmental factor. Moreover, the strength of the association is far from being negligibly small; births in the first quarter exceeded expectation by 7% for our schizophrenic patients, and by 9% for our manic-depressives (for the other diagnostic groups, the difference from expectation was about 1%). There are, of course, many non-causal explanations, the most obvious being a deviance in parental conception habits (in which case the birth pattern of the patients should also be shown by the patients' siblings). Social class differences in season of birth are not likely to be important here, for there is now good evidence that the fathers of patients with schizophrenia and manic-depression do not differ from the general population in their social class distribution^{5–7}.

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BOOK REVIEWS

Macromolecular Chemistry

Polymer Science: a Materials Science. Edited by A. D. Jenkins. Vol. 1. Pp. xxiii+1-932. Vol. 2. Pp. xxii+933-1822. (North Holland: Amsterdam and London, 1972.) Dfl. 380; \$119.

It is now almost half a century since Hermann Staudinger propounded his theory of macromolecular chemistry, and faced opposition from the might of (mainly German) classical chemists. This was not so much based on chemical fact, albeit his results were attributed to the examination of impure products, as that he had the temerity to put forward his macromolecular theory as a new branch of organic chemistry. That Staudinger's claim was if anything an understatement is amply supported by the two volumes edited by A. D. Jenkins under the broad title of *Polymer Science*. From the ample references associated with the 1,800 pages of the text, it can readily be accepted that more than half of the present chemists throughout the world work in or are associated with polymer or, more broadly, materials science.

In the preface to the first volume the editor explains that he aims to provide a "vocabulary for those who come to the subject with little previous knowledge of polymers", a statement which after performing the daunting task of reviewing the two volumes must raise some elements of doubt. Basically the work deals with those "properties of polymers which call for physical examination and upon which the performance of polymers as useful materials depends". To attain this end he has adopted what is by traditional standards a novel approach to the subject, by devoting the first two chapters (191 pages) to the principles and methods of polymer production and their chemical structure, mentioning individual materials rather as incidental illustration of basic theory. In view of the spate of literature which has appeared dealing with specific polymer families or individual members, this is to be applauded, although the level of treatment now presented is likely to tax the understanding of those wishing to obtain the general "vocabulary and

background" referred to by the editor.

The succeeding eleven chapters, which constitute the first volume (929 pages), deal with the physical properties of plastics from various aspects, ranging from the near esoteric consideration of theories of chain coiling, elasticity and viscoelasticity to plasticization which comes very near to the production area. The various physical characteristics, such as glass transition, melting point and structure; crystallinity, fracture; optical properties, and the mechanical properties both of plastics and of fibres and fibre assemblies, are separately treated. The sequence of treatment may appear illogical, but this is of small consequence since each chapter is self-contained and is virtually a monograph on the selected subject, complete with references to relevant literature. Reference is made to specific polymers mainly as illustrative of the selected property, which means that data on any material are somewhat fragmented.

The second volume begins with a relatively brief study of adhesion, considered from the viewpoint of adhesive bonding as being the force required to separate substrates that are bonded together by means of an adhesive. The theory is supported by reference to a number of commercial polymers where problems of adhesion are frequently encountered. The following chapter on "Friction and Wear" will be of interest both to the practical man and to the theorist, particularly in view of the increased importance of polymers for applications in tribology. The next chapter, on "Polymer Solutions and Fractionation", although highly theoretical, has practical significance, because polymer solutions have been largely used to evaluate problems of structure, and their solution (and in some cases fractionation) plays an important part in their commercial applications. The four chapters devoted respectively to polyelectrolyte electrical properties, and two devoted to dielectric properties, have essentially a bias towards theory, but contain useful data for the practical man in this important area of

polymer application. A more detailed study of the far infrared spectra of polymers is next given, from the viewpoint that it has long been appreciated that these data not only give an indication of the utility of polymers as transmissive media, but also details of the conformation of chains and of the interatomic forces operative in and between them. The study of nuclear magnetic resonance (n.m.r.) given in the following chapter is shown to be applied not only to basic problems of polymer structure, but also to obtaining information concerning the motion of polymer molecules. This is an area which the author rather modestly concedes as being at present "mainly in the hands of specialists". The chapter on "Degradation" is broadly interpreted as covering any sort of chemical change. The possible progressive change in the physical properties (and hence performance) of plastics materials has for long been among the major causes of their rejection by some industrial users, so that the basis of the trouble and the possibility of avoiding it are of practical importance. Closely related to this subject, and separately considered under the general heading of "Radiation Effects", is a brief review of the physical and chemical changes brought about by the exposure of polymers to high energy radiation. No references to related literature are given in this instance, which might be interpreted that the subject is now less highly rated than was earlier anticipated.

"The Identification and Analysis of Plastic Materials", a very important chapter dealing with a subject which almost defies brief treatment, might be considered as more relevant to the technology than to the science of polymers. Some aspects of the subject have inevitably been treated in other chapters, but copious references compensate for lack of detail. The next two chapters, dealing respectively with polymers for use at low and high temperatures and with composites, have the common factor that they are contributed by recognized experts in the aircraft industry, which is possibly plastics'

most exacting end-user. Even allowing for the space limitations there are some surprising omissions from the heat-resisting polymers; the organo-metallic compounds in particular appear to have been neglected. The chapter on composites is essentially technological, although there may be a difference of opinion as to where science ends and technology begins. There is certainly no doubt about the scientific nature of the final chapter on "Neutron Spectroscopy", which briefly outlines the theory of neutron inelastic scattering, or more basically of the binding forces in solids.

From this necessarily brief outline it is evident that the two volumes do full justice to the title. There is inevitably, as the editor admits, some overlapping of subject matter, but this is useful as giving viewpoints by different experts and in most cases based on different scientific disciplines. After careful consideration of the extensive text it is difficult to agree with the editor's claim that it will help anyone "encountering polymers for the first time to obtain a detailed understanding of the present level of appreciation of the nature of polymer behaviour in any selected field". Rather one must conclude that it is the book for the expert and those concerned with research and development in specific fields. The references in each chapter (classified alphabetically by author) give an excellent guide to the collected literature up to 1968/69. The indexes are adequate, but in view of the extent of the subject matter, a more detailed subject index would have facilitated quick reference. Inevitably the cost of these volumes is high, and must limit circulation even to libraries. Having regard to the specialist nature of each chapter it might have been more profitable alike to publisher and reader to split this enormous field, making each chapter (with perhaps a little judicious pruning) into a separate monograph. These volumes should at least find a place in every library concerned with polymer research.

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Nuclear Shell Guide

The Practitioner's Shell Model. By George F. Bertsch. Pp. viii+206. (North-Holland: Amsterdam and London, 1972.) Dfl. 36; \$10.50.

FOR more than twenty years the nuclear shell model has been the model on which nuclear physicists have based most of their understanding of the structure of atomic nuclei, and few of us have been happy with alternative models until some understanding of their relation to the shell model has

been obtained. There is an enormous variation in the degree of complexity of explanations of nuclear properties based on the shell model, which range from the prediction of spins and magnetic moments of nuclear energy levels from the quantum numbers of a single particle to the huge matrix diagonalization programmes for the configuration mixing of several particles. This book is an attempt to describe the middle ground between these two extremes where physical insight takes the place of elaborate calculation. The author is well qualified to write such a book, because he has done much with this approach.

The first two chapters give brief accounts of Hartree-Fock theory and the shell model, and of harmonic oscillator wave functions. The essentials of angular momentum theory and recoupling are presented in the third chapter. In the fourth chapter the nuclear interaction used in shell model calculations and its relation to the free nucleon-nucleon interaction are discussed. Chapter 5 is devoted to the effects of this interaction in nuclei in which only two particles (or a particle and a hole) are free to respond, while chapter 6 is concerned with configurations of several particles and the development of deformations of the nucleus. The next chapter deals with the effects of polarization of closed shells, which can alter both the effective interaction between particles, and their effective charges and moments. The final chapter discusses the information that can be obtained on shell structure from direct nuclear reactions by using the distorted wave Born approximation. At the end of each chapter there are problems with worked solutions.

The book is a valuable contribution to the subject, and the power and limitations of this approach should be understood by anyone who wants to work with detailed shell model calculations, or to teach students about the shell model. It is not in any sense a comprehensive guide to all aspects; many important areas are either completely omitted or barely mentioned. The classification of states by means of group theory has been very important, but there is no detailed discussion of it here. Pairing and the random phase approximation are omitted on the grounds that they are adequately treated elsewhere, but there is not even a proper explanation of the importance of short range forces for producing pairing; it might be thought from the remark made on page 98 that there is as much correlation of two particles in the state of maximum angular momentum as there is in the state of zero angular momentum. Within its limitations the book provides a clear

and concise guide to important aspects of the nuclear shell model.

D. J. THOULESS

Neutron Activation

Neutron Activation Analysis. By D. De Soete, R. Gijbels and J. Hoste. Pp. xx+836. (Wiley: New York and London, July 1972.) £14.

THAT this large and comprehensive treatise on neutron activation analysis fills a real need is evident from the constant demand for the copies in my laboratory. The volume, the thirty-fourth in the Wiley-Interscience series of chemical analysis monographs, describes within its compass of almost 900 pages most aspects of neutron activation analysis and associated techniques.

The comprehensive nature of the work is best indicated by a brief survey of the contents. Two introductory chapters are followed by a detailed account of neutron-induced reactions and then, in chapter 4, a description of the various neutron sources—reactor, accelerator and isotopic—and of their operation. The next two chapters, dealing with the radioactivity growth and decay laws, radioactive disintegration and radiation detectors, are followed (chapter 7) by a consideration of the preparation of samples and standards. Chapters 8 and 9 describe respectively activation analysis with and without post irradiation radiochemical separation and the next two chapters discuss systematic errors and the statistical interpretation of results. Chapter 12, mainly a bibliography of the field, is followed by seven appendices containing tables of cross-sections for thermal, fission and 14 MeV neutron reactions, lists of radionuclides in order of gamma ray energy, gamma ray mass attenuation coefficients and chemical yield data.

It is not to be expected that a work of this sort will be completely free of errors or omissions, but those there are are not serious. A book which considers in detail, for example, diffusion of neutrons, beta ray theory, reactor physics and so on might also be expected to describe all major relevant chemical procedures. But for some of the most common of these—ion exchange, for example—the reader is directed to other works, although these are not specified. Nor does there appear to be any discussion of the important allied technique of capture gamma ray analysis.

The index is unsatisfactory and should be made much more comprehensive in any future edition. Also the method of reference to other sections of the book is clumsy (it is best to go via the list of contents) and it should be possible in a future edition to refer by page number. The treatment of ^{252}Cf as a neutron source is too brief (six lines), while Table

12.2 listing elements determined in various matrices would be usefully complemented by another table listed in order of matrix instead of element, although this is available elsewhere, for example, NBS Technical Note 467.

These, however, are relatively minor criticisms of a book which is sure to find an important place in any laboratory engaged in the field. H. W. WILSON

Transport Kinetics

Elementary Kinetics of Membrane Carrier Transport. By K. D. Neame and T. G. Richards. Pp. xii+120. (Blackwell Scientific: Oxford, London, Edinburgh and Melbourne, 1972.) £3.

I SHOULD have liked to recommend this little book wholeheartedly. It is written in response to a definite need and the authors have successfully limited their treatment to the precise field of the title. There is, quite correctly, no discussion of the structure of membranes (although useful references are provided) and there is, again correctly, little on the properties of the "carriers". These entities are merely useful descriptions of experimental results and the book concerns itself only with the kinetic consequences of the carrier hypothesis.

The strength of the book lies in the really excellent sections dealing with the handling of kinetic data, and their analysis in terms of simple and complex carrier systems. The traps of too naive an analysis of the data and of too sophisticated an analysis of unreliable data are clearly pointed out. Most valuably, many of the examples used are wisely chosen and from a broad membrane literature, replacing the hypothetical examples that might have contented other writers. One necessarily anonymous provider of data and related "analysis" must be hiding his head in shame!

What a pity, then, that the sections dealing with fundamental theory do not match this high standard. Thus, for instance, the Michaelis-Menten equation is derived (in five laborious pages) in such a form that the concentration of binding sites appears in the Michaelis constant. The authors' terminology is often misleading, such as "association" and "dissociation" constants, where "on" and "off" constants would be more appropriate. Finally, the sections on exchange diffusion and counter-transport are completely misleading. An explicit experimental distinction between these processes has for a number of years been available. When William of Ockham (quoted by the authors in this context) wrote that "essences are not to be multiplied without necessity", he carefully included the latter words.

W. D. STEIN

Insect Natural History

Insects of the World. By Walter Linsenmaier. Translated from the German by Leigh E. Chadwick. Pp. 392. (McGraw-Hill: New York and London, November 1972.) £6.50.

In recent years there has been a spate of gaudy insect books richly illustrated with colour photographs; but in spite of the great technical improvements in colour film, in lenses, and in depth of focus, these books for some reason are apt to leave a feeling of dissatisfaction; and at the end of the day one turns with relief to the coloured copperplates of Maria Merian picturing the insects and other wonders of the tropics recorded during her journey to Surinam in 1699. Many of the same insects figure in Linsenmaier's book, depicted by hand in a different style but with something of the cornucopian extravagance that inspired Madame Merian's work. There is an abundant text, full of accurate information for those who care to seek it out. The text is illustrated by little monochrome vignettes, many of which bear a page and figure number where the same illustration can be found in full colour. The opening chapters provide simple outlines of anatomy and physiology, accounts of coloration, mimicry and camouflage, insect distribution and migration. But the main part is systematic. The insects are taken order by order, their description enlivened throughout by essays on every aspect of insect natural history. Finally, there are chapters on water insects and social insects—again systematically arranged and packed with information.

The book can therefore be read with interest, with the help of the thumb-nail illustrations, without reference to the plates. But the plates themselves are fantastic, all 150 of them; and many readers will find it difficult to leave them and get down to the text. The vast majority are superb drawings of insects in the living state in their native haunts; often crowded together in some amazonian forest or palaearctic woodland so that the child-like entomologist can pore over them again and again, and still discover something he had not noticed before. Then there are plates of massed colour photographs of cabinet specimens figuring hundreds of the more or less conspicuous insects from most parts of the world. These are so good that many can be used for identification, or approximate identification, and by looking up the pages of small print at the end of the book their generic and specific names and country of origin can be found. Among the Lepidoptera the subjects range from *Nepticula* with a wing span of 4 mm to *Attacus* with a wing span of 36 cm! A spectacular book by a splendid artist who is an enthusiastic insect lover, well

translated from the German by a distinguished American entomologist, L. E. Chadwick.

V. B. WIGGLESWORTH

EM *Vade-mecum*

Practical Methods in Electron Microscopy. Edited by Audrey M. Glauert. Volume I. Part 1. *Specimen Preparation in Materials Science.* By P. J. Goodhew. Part 2. *Electron Diffraction and Optical Diffraction Techniques.* By B. E. P. Beeston, Robert W. Horne and Roy Markham. Pp. viii+444. (North-Holland: Amsterdam and London, 1972.) Dfl. 110; \$34.50.

ELECTRON microscopy must be the interdisciplinary subject *par excellence*. The instrument itself is a relatively straightforward exercise in physics and electrical engineering, but its applications spread through practically all branches of pure and applied science, from concrete technology to macromolecular structure, from radiation damage in metals to the mechanism of muscular contraction. Further, having caught your rabbit, dissected, fixed, desiccated, stained and sectioned it into flakes thinner than one-tenth the wavelength of light, imaged it (in vacuum) in adequate illuminating conditions at a magnification up to $\times 300,000$ and enlarged the micrograph yet further, there remains the crucial task of interpreting your results, of deciding what is "real" and what artefact in the multifarious details made visible. The metallurgist has a like problem in preparing his specimens and analysing his images of dislocations or precipitates, though these are less liable to artefact.

Twenty years ago it was possible, within the compass of 300 pages, to produce a fair survey of the subject, from the principles and operation of the microscope through specimen preparation to electron photography. True, the author (if solo) needed to be something of a jack of all trades to provide a reasonably balanced view of every topic, but the early electron microscopists had to be just that, serving as they usually did customers from various departments of a university or research centre. Even 7 years ago one man could still cope adequately with the *Elektronenmikroskopische Untersuchungs und Präparationsmethoden* in 600 pages (Springer-Verlag; 1967), although Dr Reimer had to rely on help and advice from numerous colleagues as the preface acknowledges. The French a few years earlier required two volumes totalling nearly 1,300 pages and a team of seven authors (*Traité de Microscopie Électronique*, Hermann, Paris; 1961), but gave more space to the microscope itself.

By the 1970s it is no longer possible to give an adequate treatment even within two such volumes. Quite apart from the natural spread of applications, major new instrumental developments continue to surprise us, such as the scanning transmission microscope, and new insights into image formation have allowed the heavier atoms to be "photographed". In this situation only a collaborative effort by a group of experts can do justice to the subject. A German scheme breaks it down into many small sections, which appear seriatim as each text is completed, to be bound in loose-leaf form (*Methodensammlung der Elektronenmikroskopie*, Wissenschaftliche Verlagsgesellschaft, Stuttgart; 1971).

An English enterprise in rather different form has now been launched, edited appropriately by a physicist now researching in a biological laboratory. Dr Glauert is assembling a team of authors to provide a "comprehensive series of laboratory handbooks in which all the techniques of electron microscopy are described in sufficient detail to enable the isolated worker to carry them out successfully". The first volume certainly lives up to this aim. It has two parts, the first of which deals with specimen preparation in materials science. The chief preparative techniques for metals and other solid samples, including powders and fibres, are described by Dr P. J. Goodhew in 189 pages. Part 2 is in two sections, an introduction to electron diffraction by Dr B. E. P. Beeston (140 pages), followed by an account of the application of optical diffraction and image reconstruction techniques to electron microscopy by Professor R. W. Horne and Professor R. Markham (110 pages).

In selecting the authors the editor has obviously followed the rule enunciated in her preface that "it is not possible to describe a technique with sufficient practical detail for it to be followed accurately unless one is familiar with the technique oneself". Each contribution is well planned and clearly presented, starting from elementary principles and working up to the present state of the art. The text is illustrated with diagrams, charts and micrographs; references are given after each chapter and a list of suppliers of relevant equipment and materials at the end of each part. A subject index is also provided for each part, as the primary intention of the editor and publishers is for individual parts to be available in paperback form. In the same way volumes II and III are announced as each comprising two rather unrelated parts. So that what we are offered is a series of monographs, from which the advanced student or research worker can select those he needs on his work bench. The hardback edition appears to be provided

mainly for the convenience of libraries. This is an imaginative venture which deserves to succeed, if the standard of the first two parts can be maintained. It is a little disappointing, however, that the price of the first paperback parts is still relatively high (about £4 and £5.50), almost two-thirds that of the bound volume I.

V. E. COSSLETT

Peaceful Revolution

Management and Technology. Edited by Alan Mencher. Volume 1. An Anglo-American exchange of views. (Inforlink: Frimley, Surrey, 1972.) £3.80.

WILL this be the "century of the mismatch", in which our technological power outstripped our strength of moral purpose, and our social institutions—designed for an earlier environment—proved unable to modify themselves quickly enough to tackle the right problems? Alan Mencher's selection from the discussions which he has so successfully organized at the US Embassy provides some answers. Blake, Ackoff, Ramo, Charpie, Schon and others—in talk and discussion with their British audience—show that the Americans have moved from the consideration of problems as isolated discrete issues to the position of studying them as part of the larger system which is their environment. They still lack the tools, concepts and experience for tackling complex systems problems but the systems approach, however imperfect, does force people to set down their goals and the criteria against which proposals can be judged.

Society, by creating an increasing need for a priority selection of satisfactions, is forcing political action and together these provide new opportunities for industrial endeavour. New ideas mean change, and rapid response to change may often only be achieved by revolution. The key problem in innovative management is how to produce peaceful revolution so that every individual's competence can be fully exploited. American business has reacted by, as Schon puts it, moving from the concept of a firm producing a highly aggregated product to that of the firm conceived as an organization built around a process as its defining character. This notion can best be seen in the constellation firm, such as 3M, in which semi-autonomous firms surround a bank and a development facility. Such a firm is highly adaptive—satellites can expand, contract or be replaced. Flexibility like this in industry imposes great strains on other institutions, particularly in research and education. These have to adapt to a faster internal rate of change, so that they meet more readily the real

and continuing needs of firms and individuals alike.

One mis-match is very noticeable in this series of papers. These American notions of management have moved well ahead of ours. A contribution from a representative of one of America's besieged or decaying industries would have introduced an interesting counter-balance, for not all would agree that rapid change is necessary or desirable. As we move closer into the European community, the clash of cultures in the problems of reconciling social and technological developments will become more devastating in their effect. The Americans have the advantage of being able to experiment on a larger scale than we can afford. Their search for solutions, as revealed in these papers, provides a valuable starting point for those of us who are beginning to recognize the nature of the changes in industry, government and educational systems which will occur during the rest of this century.

E. P. HAWTHORNE

Molecular Concepts

Atomic and Molecular Structure: The Development of Our Concepts. By Walter J. Lehmann. Pp. xix+449. (Wiley: New York and London, July 1972.) £5.50.

THIS book represents a courageous assault upon an important problem: how to teach aspects of a highly technical and mathematical science to people without a scientific background. In this case the subject is atomic and molecular structure and the students were those reading for non-science majors at Boston. Five main sections deal respectively with developments leading up to the periodic table, the evolution of the nuclear atom, elucidation of the electronic structure of the atom, quantum mechanics and molecular structure. One imagines the liberal arts students would turn often and gratefully to the appendices on the metric system and "a quick refresher on arithmetic procedures", to say nothing of the very full glossary.

The value of Professor Lehmann's work can only be assessed in practice, but it has the marks of sound educational strategy. Little is left to chance, the text is clear and interesting, there are some useful graphics, and self-assessment questions monitor the individual's progress. There are a few mistakes in formulae, equations and calculations, but they are not likely to be serious impediments.

In place of the conventional rigorous treatment of his themes the author adopts an approach that may be fairly termed quasi-historical. There is no

attempt to give a complete historical coverage but each section has a broad historical framework. As he says, "we are interested in the logical development of ideas rather than tracing their history". Such an approach can be fraught with hazards, and it says much for the author that many of the most obvious pitfalls are avoided. Certainly we have Galileo at the Tower of Pisa, Newton's apple and a few other items from scientific mythology but these tend to be confined to the early centuries. More serious is the "whiggish" view of historical progress that is implicit in much of the book. The unfolding of science is seen as having "all the elements of a good detective novel" with "a solution of the mystery through sheer perseverance". But of the non-rational elements in scientific development, of the false starts and of the brilliant "hunch", little is said. This would not matter so much were it not the avowed aim of the book to emphasize how the scientist develops his theories and how he approaches a problem.

As a reliable historical guide, therefore, this book is inadequate. But it may well turn out to be a valuable companion to those concerned to communicate modern science within a non-scientific culture. C. A. RUSSELL

Unravelling Interactions

Migration of Interacting Systems. By L. W. Nichol and D. J. Winzor. Pp. x+166. (Clarendon: Oxford; Oxford University: London, November 1972.) £5.50.

BIOCHEMISTS classify electrophoresis, sedimentation and gel chromatography as migration processes. If these methods are used straightforwardly for separating biological macromolecules, only a relatively simple grasp of underlying principles is required, as long as the migrating material is confined to a thin zone. Nevertheless, this simplicity is paid for by a loss of information about macromolecular interaction, a matter of strong topical interest.

If the zone is made broad with a plateau of constant concentration intervening between its extremes, the conditions for interaction are preserved. Qualitative indications of interaction may then be obtained by comparing leading and trailing boundaries. Even at this level not much depth of theory is required. It is in moving to a quantitative measure of interaction that a new dimension of difficulty emerges.

In part this difficulty is inherent. Complexes have to be studied under conditions determined by the circumstances in which they are stable, ruling

out such usual simplifications as extrapolation to zero concentration. Indeed, measurements have often to be made at sufficiently high concentrations for non-ideality factors to be dominant. Quite apart from this, very complicated effects of re-equilibration and diffusion within the boundaries need to be understood. Consequently a biochemist finds himself in the situation he has met before over X-ray crystallography and nuclear magnetic resonance spectroscopy, where he can feel himself diverted completely from his subject of study in order to master the tools needed to pursue it.

It is here that a monograph of the right kind could lead to a great economy of effort. Biochemists are likely to agree that for them this means one which begins at a very simple level with an account of the basic theory of interacting systems, but which then takes them step by step to a point where they are able themselves to deal with real systems.

The most recent advances turn out to be quite helpful in this respect, for much of the forbidding complexity of interaction theory has been due in the past to attempts to find analytical solutions of the basically simple flux equations. It is now quite obvious that all this can be by-passed and that answers of any desired precision will be obtainable by numerical methods without having to lose sight of the underlying physical situation and without having to neglect non-ideality factors, diffusion effects, pressure effects or any factors which can be clearly defined. A distinction can then be made between the initial very difficult job of writing the necessary computer programs to carry out the numerical work, and the subsequent use of these programs to analyse experiments.

This monograph does not adopt this point of view. In particular the introduction is very difficult for beginners to whom, after all, the editors address their monographs. Perhaps the authors felt that the well-known review article by Nichol *et al.* published in 1964 already provides a sufficient introduction. So readers have to do a great deal of hard work right from the first page, including mastering very general ideal cases of which a self-associating acceptor interacting with a self-associating ligand is a key example. One soon realizes that the book is patterned on the thirty or so papers written over the last ten years by the authors, and as they say in their preface, their choice of examples is "guided by the interests of the authors in protein research".

Even so, most areas of interest are touched upon, for example monomer-polymer systems in rapid equilibrium, and related "sigmoidal" ligand binding, protein-protein association and hybrid-

ization, protein interactions with buffer constituents, frictional effects and charge effects in sedimentation. Also considerable attention is paid to the quantitative side of gel chromatography in which the authors have played a prominent part. For those familiar with the subject, it is particularly useful to have an exposition summarizing the authors' point of view in a subject in which there remain many areas of controversy. One of these areas is clearly that of the effect of pressure on the sedimentation of myosin, for one notices that the authors' interpretation contradicts that of the American editor of the monograph who made the original discovery of this effect. G. A. GILBERT

Hormones on the Brain

Steroid Hormones and Brain Function. Edited by Charles H. Sawyer and Roger A. Gorski. (Proceedings of a Conference held in May 1970, California.) Pp. 388. (University of California: Berkeley, Los Angeles and London, 1971.) £13.50.

ABOUT fifty authors contribute thirty-two papers to these proceedings of a conference sponsored by IBRO and by the UCLA Brain Research Institute and Forum in Medical Sciences. The hormones of the book's title comprise those of the adrenal and gonads; brain function is appraised by electrophysiological and behavioural characteristics and elucidated by a variety of techniques including metabolic and histochemical ones and the placing of surgical lesions.

It is encouraging that in the brain, as in peripheral target organs, attachment has been found of steroid to cytoplasmic and nuclear components. W. E. Stumpf, basing his observations on the cerebral occurrence of oestradiol-binding proteins similar to those of the uterus, reports autoradiographic localization of the cells concerned. After systemic administration of [³H]-oestradiol to rats, uptake was found to defined neurones of the diencephalon and amygdala, and not to glial and ependymal cells. It is suggested that the neurones concerned produced the pituitary secretions which in turn acted on the peripheral target organ. R. A. Gorski concludes, from implantation of actinomycin D in the hypothalamus of rats, that oestrogen action in inducing aspects of female sexual behaviour proceeds through RNA and protein synthesis. The RNA content of cerebellar explants is shown by A. Vernadakis to be increased by both cortisol and oestradiol, while B. S. McEwen describes excellently the differential localization of corticosterone and oestradiol in the rat brain.

In his "overview" of the conference,

J. M. Davidson queries how much of the behavioural effects of steroids occurs by action of the steroid at the brain itself—and answers: part only. With each well-investigated steroid, evidence was offered for direct action at the central nervous system, and further actions from peripheral organs. It is thus valuable that measurements can often be made in small quantities of blood of the relevant hormones. V. D. Ramírez describes determinations of luteinizing hormone and follicle-stimulating hormone in 0.2 ml. of plasma from rats by radioimmune assay.

The book carries prominently both behavioural and electrophysiological consequences of steroid actions in developing and in adult animals. Most papers are well-documented, but with figures awkwardly placed for consultation: not in the relevant text, but following it. Production and indexing of the book are good, and its discussion sections are plentiful and informative.

H. McILWAIN

Fruit Fly

Drosophila. By Bryan Shorrocks. Pp. 144. (Ginn: London, October 1972.) £2.25.

Drosophila melanogaster is once again a popular beast in biological research laboratories, especially those devoted to developmental and biochemical genetics; it never did lose its popularity with the population geneticist.

This text by Dr Shorrocks will be useful to anyone starting to work with *Drosophila* since it provides a succinct and clearly written guide to general biology, laboratory culture, field ecology, methods of collecting, behaviour and elementary cytology. There is also a section devoted to elementary genetics with some suggestions for experiments suitable for the beginning student, but the most valuable single feature is the illustrated diagnostic key and ecological notes relating to a score or so of the commoner species found in Britain. It is ironic that even for species which have been widely used in genetic studies, little is known about their primary breeding sites, population sizes, fluctuations in numbers, distribution, and so on. The British fauna includes a group of closely related species like *subobscura*, *ambigua*, *tristis*, *obscura* and *subsilvestris*, whose population biology would certainly repay study. No doubt their similarity in appearance has hitherto often proved discouraging, but Dr Shorrocks's key and clear line diagrams have removed this obstacle and should stimulate comparative studies on the population ecology of these and other British species.

F. W. ROBERTSON

Seeds

Seed Biology. Edited by T. T. Kozłowski. Volume I. *Importance, Development and Germination*. Pp. xiii+416. (Academic: New York and London, May 1972.) \$26.40.

THE first volume of *Seed Biology* is subtitled *Importance, Development and Germination*. It is part of a three-volumed work, the second of which will cover germination control, metabolism and pathology; the third will deal with applied aspects of the subject including insects, seed collection, storage, testing and certification. This first volume, then, is really meant to be seen as part of a larger work, and this is made clear by several references within its six chapters to material in subsequent volumes.

The whole work is introduced by T. T. Kozłowski (the editor) and C. R. Gunn in a short chapter dealing primarily with the significance of seeds in evolution, their great diversity and their importance to man. This is followed by two chapters on the development of seeds, concentrating mainly on anatomical aspects; H. Singh and B. M. Johri consider the gymnosperms and S. P. Bhatnagar and Johri the angiosperms. Both are worthy accounts which distil an enormous amount of information from a large number of references—nearly 350 in the case of angiosperms.

The fourth chapter by A. Fahn and Ella Werker is an interesting and straightforward description of the anatomical mechanisms of seed dispersal and, in spite of the large number of cases considered, they conclude that the principal types of device serving seed dispersal are quite small. The style and objectives of chapter 5 on the morphogenesis of seed germination by G. P. Berlyn contrast with the rest of the book. Instead of attempting an encyclopaedic coverage, the author has decided to take two contrasting genera, *Zea* and *Pinus*, and give a critical (and very readable) consideration which relates the anatomical and sub-cellular changes in seeds to the wider problems of morphogenesis.

Finally there is a chapter by B. M. Pollock and E. E. Roos on seed and seedling vigour. This is a wide-ranging account of a difficult subject—a difficulty which arises in part because of the lack of precision often attached to the term "vigour". Nevertheless, there are some interesting ideas here.

One cannot form a final judgment of a volume which is only one-third of a complete work, but there is little doubt that seed research workers will find it a useful reference book. Most chapters are designed primarily for a specialist audience, in spite of the publisher's blurb; it is unlikely, for example, that

they will be of interest to seed growers, as is claimed. The production is very fine and the illustrations are good and lavish—and so they should be, considering the price. I shall look forward to seeing the subsequent volumes.

E. H. ROBERTS

Operational Research

Operational Research: Techniques and Examples. Edited by G. H. Mitchell. Pp. ix+275. (The English Universities: London, 1972.) £3.95.

THIS book is based on material used in the training programme of the Operational Research Executive of the National Coal Board, a programme now mounted jointly with Brunel University as a Master's Course, and follows the publication of a similar book¹ some ten years ago.

It contains chapters on all the major techniques associated particularly with OR: linear programming and its extensions, dynamic programming, decision theory and theory of games, networks, queues, stock control, replacement, and simulation. As such, it does not differ greatly from many other introductory books on OR, although the editor has managed to pack a lot of material into the 200 or so pages. Brief descriptions of many extensions of the basic techniques are given (for example, post-optimality analysis and parametric programming, decomposition, stochastic programming, separable programming, integer and mixed integer programming are covered in fifteen pages), together with further references, which will provide a good basis for an introductory course, but which will be tough going for the unsupported reader.

The editor has aimed to keep the mathematical level within the reach of anyone with "A" level mathematics, but the liberal use of algebraic notation is likely to make comprehension difficult for the reader who has not progressed beyond that level.

Each chapter is provided with a number of problems and worked answers, amounting to some fifty pages in total. The book is certainly a good candidate for selection as the main reference for an introductory course on OR.

ROBERT HARRIS

¹ Houlden, B. T. (editor), *Some Techniques of Operational Research* (The English Universities Press, 1962).

Addendum

IN the bibliography to the review of the book, *The Careless Technology* (*Nature*, 240, 268; 1972), the publisher was given as The Natural History Press: Garden City, New York. The book is also published by Tom Stacey, London.

CORRESPONDENCE

Coal

SIR,—In your article, "The Biggest Lame Duck of All" (*Nature*, **240**, 431; 1972), you make comments about the wisdom or otherwise of the measures now proposed for the British coal industry in the next few years. Your comments cover a range of topics from energy policy to the philosophy of control of the nationalized industries. These views will no doubt be given an appropriate place in the considerations of responsible individuals, including those who are concerned with the parliamentary stages of the Bill.

However, towards the end of the article, you ask why the new proposals have not been accompanied by some policy on research, a subject on which *Nature* would be expected to be well informed. I hope your readers will not be led to assume from this question that the National Coal Board lacks appropriate policies on research, especially in the field of future utilization technologies for coal. The Board's policy on research is not only a matter of prime concern to the Board itself but the appropriate Minister also has a statutory duty to satisfy himself that the research programmes of the NCB, along with those of other energy industries, are satisfactory. The Minister is advised on this by an advisory body having an eminent and expert independent Chairman and membership.

Furthermore, the Board has always made a great point of explaining its attitude to future developments in the role and modes of utilization of coal. Indeed the current Bill owes much to the growing acceptance not only that coal provides much the greatest reserve of fossil fuel but also that coal is capable of being used in a flexible fashion to replace other fuels, including liquid and gaseous fuels. The Board's research establishments are open to display the research work and to explain these policies. The laboratories have been regularly visited by numerous representatives of the press, government organizations, other related energy industries, and visitors from many countries. We have, incidentally, also made a point for a long time of presenting our proposed research and development programmes, before they are finally approved, annually to our National Consultative Committee, which is the representative body of our trade unions; this body also visits our laboratories and takes a deep interest in the work. The Board's research work and policies have also been widely publicized through lectures and articles in both professional and popular journals.

The consensus of international opinion certainly seems to indicate that the Board's current programme is sensible and that our longer range planning will allow us to develop our strategy as our opportunities expand, as we believe they will.

Nonetheless, the Board would still be willing to demonstrate its research work to *Nature* and to explain the reasons behind its policy. It would seem desirable that you should understand the facts before criticizing the policy of the Board, and indeed the Government, in this regard.

Yours faithfully,

L. GRAINGER

National Coal Board,
Hobart House,
Grosvenor Place,
London SW1

Developing Medicine

SIR,—The Cameroon Embassy would like to draw readers' attention to the fact that in addition to the training of highly qualified medical personnel (doctors, nurses, and so on), the University Centre for Health Sciences (CUSS) in Yaounde, Cameroon, also trains a large number of para-medical staff. In fact the CUSS complex is unique in Africa in that training involves all aspects of medical and sanitary sciences.

Nevertheless, the use of a large number of para-medical personnel should be and must be considered as a very temporary solution to health problems in developing countries. The ultimate aim is to have regular highly qualified medical personnel as soon as possible in as many medical and health centres as possible with the para-medical trained staff serving as complements to those centres.

In the colonial days, emphasis was laid on curative medicine only and well equipped hospitals and personnel. All these demanded a lot of money and technical assistance which was scarce in penurious developing countries.

The Government of the United Republic of Cameroon, aware of this situation, has reversed this policy and has rather laid more and greater emphasis and priority on preventive medicine. In this regard, in the current Five-Year Development Plan of Cameroon, priority has been given to the construction, extension and equipping of provincial hospitals; quantitative and qualitative improvement of the training of medical staff; development of preventive and curative medicine and a vigorous health education scheme for the masses.

The end result therefore would be

the extension of health services, including all forms of medicine, to cover the entire population both urban and rural. This will include treatment, prevention, education, etc. It is intended to build 30 divisional and sub-divisional hospitals and to construct or develop 165 rural health centres in Cameroon.

Thus the Development Plan of Cameroon is aimed at achieving equitable distribution and diversification of goods, services and human resources. Bilateral and multilateral aid is welcome but the ultimate goal is to drive towards self-reliance.

Yours faithfully,

FRANCOIS-XAVIER TCHOUNGUI
Ambassade de la Republique Unie du
Cameroun,
Washington DC

Vietnam Bombing

SIR,—Your editorial on consequences of the bombing in Vietnam (*Nature*, **241**, 1; 1973) solves no problems but raises unnecessary puzzles of its own.

First of all, I cannot see how the hypothetical effect on the bombings on the coming Kennedy Round of tariff reductions, or Dr Kissinger's dilemma (if it exists) in deciding between the White House and Harvard comes closer to "Nature's parish" than "The Vietnam war as such". Where, one wonders, can one find a reader of *Nature* who would not see more pertinence in questions such as "the long-term effects of the bombing, the use of CS and of herbicides and other methods of defoliation, both on the people and the ecology of Vietnam" which the AAAS now wants the National Academy of Sciences to investigate (see page 7 of the same issue)? For that matter, the lovers of nature I know would even take an interest in the short-term effects.

Secondly, where can we find the European statesman whom the "irrationality of the bombing over Christmas" has so boosted morally speaking that he felt free "to talk as an equal to the United States"? He seems spectacularly absent in the major European countries (England no exception), whereas Palme of Sweden and Jørgensen of Denmark have a prior record of not having needed that boost.

Thirdly, who among suffering mankind, other than Dr Kissinger himself, are you now prepared to take in as your parishioners?

Yours faithfully,

MORTEN SIMONSEN
The University of Copenhagen,
DK 2100 Copenhagen

Obituary

Sir Ronald Baskett

SIR RONALD BASKETT, who died on November 24, worked for almost the whole of his career in agricultural education and research. After graduation from Reading his appointment in 1924 as assistant to the Head of the Chemical and Animal Nutrition Division of the Ministry of Agriculture for Northern Ireland afforded him an opportunity to extend one of his important interests, namely, the application of the techniques of analytical chemistry to the study of the composition and nutritive value of foods for man and livestock. At Queen's University, Belfast, where he worked until 1959, he took part in early investigations of mineral metabolism of farm animals and on the quality of products such as bacon and eggs. This was at a time when agriculture in the province was becoming aware of the value of producer organizations in the marketing of produce and in the maintenance of quality so important to exporters to the mainland. Though at this time, and indeed subsequently in his career, he did not have much time to devote to his own research because of heavy administrative duties, Baskett succeeded in building up an enthusiastic group of research workers at Queen's which made considerable contributions to knowledge of the nutrition of pigs and poultry and the utilization of forage crops and the interest in these topics is still maintained in the Agricultural Chemistry Department.

He was appointed Head of the Chemical and Animal Nutrition Division in 1928 and Professor of Agricultural Chemistry at Queen's in 1935, helping in building up the Faculty of Agriculture

and later acting as Dean for a considerable period. He had a great interest in course structures in agricultural science teaching and served as a member of the Inter-University Council for Higher Education in the Colonies. In 1950 he left Belfast to spend a two-year period as Agricultural Attaché to the British Embassy in Washington, returning to become Permanent Secretary to the Ministry of Agriculture in Northern Ireland, but he only remained in the post for a short period, returning to the University as Professor and the post of Chief Scientific Officer in the Ministry which he had held since 1947.

Baskett's long experience of university and government administration in science was of great value to him in 1959 when he undertook the onerous task of Director of the National Institute for Research in Dairying. Here he contributed greatly to the development of closer links between the University of Reading and the Institute. He recognized the value of these links in the applied sciences associated with agriculture. At Reading he took a particular interest in areas of research of immediate benefit to the dairy industry such as the development of husbandry procedures for the control of mastitis.

In addition to his day-to-day management of the Institute, Baskett also served on many committees, for example on milk composition and on the veterinary profession. He was Chairman of the ARC Working Party on the Nutrient Requirements of Pigs which published a report incorporating new recommended feeding standards for this animal in 1967.

He retired from the Directorship of the National Institute for Research in

Dairying in 1967 and returned to live in Northern Ireland. It was a measure of his dedication to agricultural research that even after his retirement he was persuaded to undertake the Secretaryship of the ARC as a short-term appointment in 1971. During his year of office he took care of the negotiations between the Council and the Government Departments on science research policy and this work contributed greatly to the development of future arrangements for research management in this field.

He was awarded the OBE in 1947, received an Hon DSc from Queen's University, Belfast, in 1963, and was knighted in 1966.

He is survived by his wife and three sons.

Errata

PARAGRAPH five of the News and Views article "Age and Cancer Incidence" (*Nature*, **241**, 238; 1973) described experiments in which tissue transplanted from cancer-susceptible animals into various hosts was found still to be cancer-susceptible when, long after the original operation, a carcinogen was applied to the transplant. Conversely, cancer-resistant tissue remained cancer-resistant even when transplanted into a cancer-susceptible animal. Unfortunately, the News and Views article inadvertently stated the opposite of this result.

In the article "Terrestrial Heat Flow, the Neutrino Problem, and a Possible Energy Source in the Core", by V. M. Hamza and A. E. Beck (*Nature*, **240**, 343; 1972) the last figure in column 7 of Table 1 should be 0.71, and not 0.11.

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